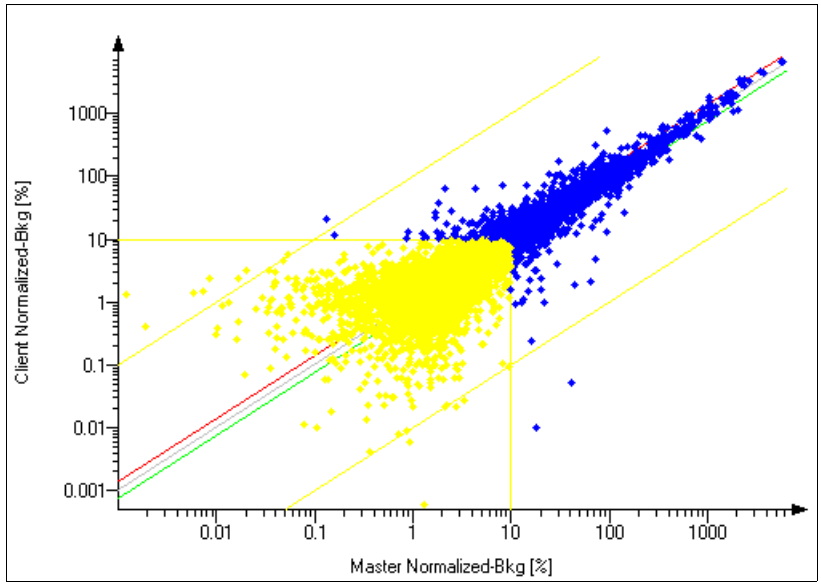
raytest

AIDA Array Compare

User's Manual



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Introduction 1

Overview

AIDA Array Compare is intended for comparing two arrays, that were both obtained by AIDA Image Analyzer's Array Metrix module.

AIDA Array Compare provides a set of visual tools, allowing you to display the two arrays in question as well as the result of the comparison in graphical and/or tabular form. You can compare arrays using schematic views, correlation plots or data tables, which can be configured by the user according to their needs. All data views are interlinked, meaning for instance, that two arrays can be moved, zoomed and positioned simultaneously.

System Requirements

AIDA Array Compare's minimum system requirements are as follows:

- Intel Pentium compatible CPU
- One of the following operating systems:
  - Microsoft Windows 9x/Me
  - Microsoft Windows NT 4.0
  - Microsoft Windows 2000:

Table 0-1 Memory Requirements

	Windows NT 4.0	**Windows 2000**	**Windows XP**
minimum	128 MB	128 MB	256 MB
recommended	≥ 256 MB	≥ 256 MB	≥ 512 MB

- CD-ROM drive
- Graphics adapter capable of displaying at least 1024 by 768 pixels in High or True Color mode
- 17" color monitor (21" color monitor recommended)

Installing AIDA Array Compare

Follow these steps to install AIDA Array Compare.

1. Insert the CD-ROM in the CD-ROM drive on your computer.
2. Choose **Run** from the Windows **Start** menu to display the **Run** dialog box.
3. Then type the following and click **OK**:
   "*drive*:\Aida Array Compare\Setup.exe"
   where *drive* is the CD-ROM drive identifier.
4. The install wizard guides you through the installation process. Click **Next** to proceed through the steps.

The **Setup** program installs the AIDA Array Compare program on your hard drive and creates a new group called **Aida** in the Windows **Programs** menu of your taskbar. You can start AIDA Array Compare by selecting it from the **Aida** cascading menu.

Do not attempt to merely copy the files on the disk, since this will not result in a successful installation of the program.

Note – To execute AIDA Array Compare, read and write permissions for the Aida-Config folder must be set for all users.

Note – The user's manuals are provided in Adobe PDF format. To open the manuals from inside the program via the **Help** menu, Adobe Acrobat Reader must be installed on your system. If not, please install Acrobat Reader. A copy of Acrobat Reader is included with the installation CD (in the folder Redistribution\Acrobat Reader).

Dongle Protection

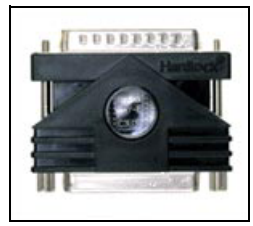


Figure 1-1 Dongle Hardware Key

The full version of the AIDA software is protected by a dongle hardware key. Before you can start the program, you have to plug the dongle into your parallel port. If your printer cable is also attached to this port, turn on the printer before starting AIDA. This will avoid possible problems in recognizing the dongle by AIDA.

Other problems in recognizing the dongle can be caused by further dongles attached to your system. In this case be sure that the AIDA dongle is the first one on port. Since AIDA dongle is fully transparent it does not effect the other dongles.

Note that there is only one dongle for all AIDA products, so there is no piling up of AIDA dongles at your parallel port.

Licensing Key

If you have downloaded AIDA Array Compare from our website, you need a license number to unlock the software. When you install the software, an hardware installation-specific serial number is automatically generated. If you start AIDA Array Compare the first time, a dialog box appears, asking you to enter your license number. To get this licensing key, you must register your AIDA copy by contacting the `raytest` customer service online or via phone/FAX specifying the serial number given in the **Serial #** text box of the **License** dialog box. If you choose to register online, complete and submit the web registration form. You will get your licensing number via e-mail within a few days. The obtained license grants you a trail period of 30 days during which the software is full functional. After the trial period has expired, you have to purchase the software in order to carry on using it.

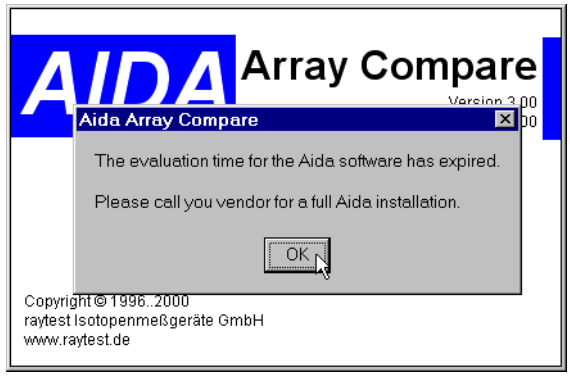


Figure 1-2 License Expired Message Box

On purchase you get the program CD containing the purchased AIDA application modules together with a dongle hardware key (see above) and a permanent license number required to unlock the software.

Problems

Some problems might arise during the use of AIDA. Please check first if your computer system is running smooth with regular software, like word processors or graphical programs. If the problems continue though other software runs fine, we would like to know about your problems. Please report the problem together with your hard and software settings to raytest, so that we will be able to fix it in the next update of the program.

Using Array Compare 2

Program Structure

The AIDA Array Compare program is a typical Windows program. All standard Windows functions, like moving and re-sizing windows, clicking buttons and using toolboxes are exactly as described in the Windows manual.

To keep this manual short, all functions that are generally used by Windows programs are not explained in detail. This manual assumes that you are familiar with Windows.

The term 'clicking' always implies the use of the left mouse button. The use of the right mouse button will be explicitly mentioned.

For multiple selections, first click an item, then hold down the Shift key while selecting another one to ensure that all items between those two in the list are selected. To select two or more non-adjacent items, click an item, then click another one while holding down the Ctrl key. Holding down the Ctrl key and clicking an item a second time will deselect it.

Menus and toolbars allow a user to interact with the AIDA program. Most functions are accessible by clicking the appropriate button on the toolbar as well as by clicking the appropriate item on a menu. Both, the toolbar and the menu, change depending on the active window and toolbox within the AIDA program.

Functions activated by buttons from the toolbar or from menus always affect the currently active window. You can distinguish the active window from the inactive ones by the different color of the title bar. Just click on the desired window to make it active.

Current messages are displayed in the status bar at the bottom of the screen. The appearance of the status bar also depends on the active window.

AIDA Array Compare is a modular program. In the **Evaluation** menu you can choose from the available functions, whereas the basic functionality is build into the integrating software.

Whenever you switch from one evaluation to a another one, the results of the actual evaluation will be stored in the Array Data File before a new evaluation can be started.

Data Structure

All information processed by AIDA Array Compare is based on array data obtained by AIDA Array Metrix. Therefore, the only available file format for loading array data is the AIDA Data Format (.adf). This file format is generated by AIDA Array Metrix and contains all information related to the array and its underlying original image data (overlays, image representation, result data, etc.) AIDA Array Compare stores its comparison data in a separate file using its own AIDA Array Compare (.ary) format. The original array data are left unchanged. The array comparison data file contains no raw image data, but symbolic links to the respective image files. Therefore, if you want to move the comparison data file together with the corresponding image files to another location, you have to move the image files manually using the **Windows Explorer**. Irrespective of the directory paths saved in the comparison data file, Array Compare uses the current path of the Array Comparison Data file in the first place. If Array Compare cannot find any image files, the path saved with the comparison data will be searched instead. If this is not successful, the search path specified via the **Image Search Path** entry of the **Option** menu is tried. If there are still no image files found, the user is prompted for the path.

On loading array files into Array Compare, the program copies all the relevant array data (except for the raw image data itself) for comparison and saves it later together with the comparison results to the .ary file, so the array .adf files becomes obsolete after saving your array comparison results.

To review the image and evaluation file information for the current data set, select the **Information** option from the **File** menu.

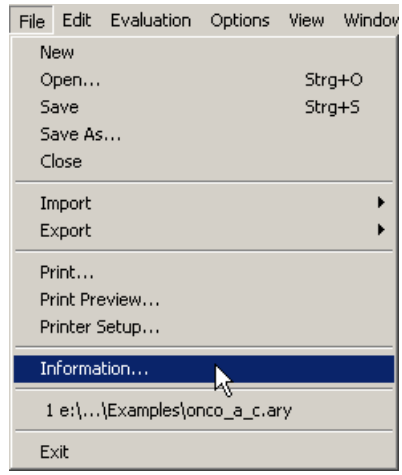


Figure 2-1 Selecting Information Command from File Menu

This opens the **File Information** dialog box.

The **File Information** dialog box consists of three tabbed pages. The **Master Array** tab displays all information stored in the ADF file of the master array. This includes information about the image, the underlying raw image data file, the parameter file, the measurement, the device that created the image as well as information about the array evaluation file together with comments entered there. The path of the files, the date when the original image was created, and the image resolution are shown. For scanner images the image size, resolution, pixel size, and (depending on the data format of the image) the file name originally used to store the scanned image are given. However, for camera images only the dynamic range and the resolution are given. The dynamic range (here 65536 gray scales) and the total size of the image are displayed. Information about the measurement and the device that created the image might be omitted if the device or image format does not support further exposure information.

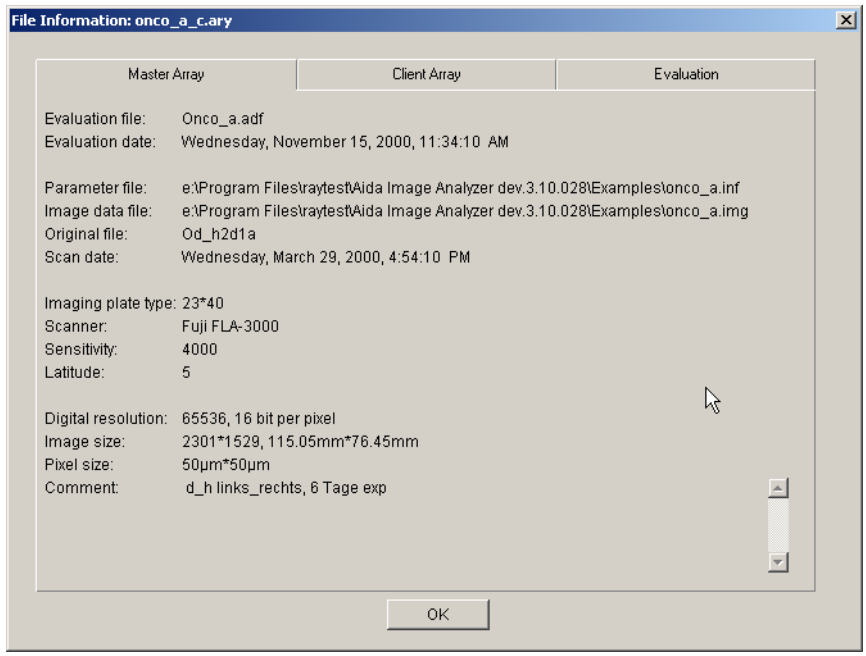

File Information: onco_a_c.ary
Master Array
Client Array
Evaluation
Evaluation file: Onco_a.adf
Evaluation date: Wednesday, November 15, 2000, 11:34:10 AM
Parameter file: e:\Program Files\raytest\Aida Image Analyzer dev.3.10.028\Examples\onco_a.inf
Image data file: e:\Program Files\raytest\Aida Image Analyzer dev.3.10.028\Examples\onco_a.img
Original file: Od_h2d1a
Scan date: Wednesday, March 29, 2000, 4:54:10 PM
Imaging plate type: 23*40
Scanner: Fuji FLA-3000
Sensitivity: 4000
Latitude: 5
Digital resolution: 65536, 16 bit per pixel
Image size: 2301*1529, 115.05mm*76.45mm
Pixel size: 50µm*50µm
Comment: d_h links_rechts, 6 Tage exp
OK

Figure 2-2 File Information Dialog Box (Master Array Tab)

On the **Client Array** tab the same type of information as for the master array are given for the client array.

The **Evaluation** tab contains information about the array comparison evaluation file (.ary). Comments can be added for future reference.

Overlays

Overlays are regions on the image as well as annotation defined within AIDA Image Analyzer and its modules. They are saved together with other evaluation data in the associated evaluation file (*.adf) as explained in the Data Structure section above. Since AIDA Array Compare compares arrays previously evaluated within AIDA Array Metrix, there is no need to create new overlay or edit existing ones. Consequently AIDA Array Compare provides no functions for creating or editing overlays. You can only select overlays to activate them.

When an evaluation is performed on the activated overlays, the created data can be visualized in other windows like the **Array Result Table** window. The data corresponding to activated overlays are highlighted in these windows.

Clicking beside an activated overlay or activating another overlay deactivates the previously activated overlay. Its color changes to blue.

Clicking an overlay or dragging a frame with the left mouse button activates one or a group of overlays.

Main Menus

When an file is open, the following main menus are accessible:

Figure 2-3 Main Menu

Note – Many menu items can be directly activated through the corresponding button and connected toolbox in the toolbar (see the Toolbar Structure section for details). The **Window** and **Help** menu are general Windows features and will not be further explained. So far, no context-sensitive help or tool tips are available.

File Menu

You use the **File** menu to perform general data transfer actions like creating, loading, saving and exporting array comparison, templates, and reports, printing images and results, reviewing image information, and exiting the program. With the exception of the import and export functions, it handles the entire data set of an image at the same time.

Figure 2-4 File Menu

New is used to create a new comparison between arrays. You can compare two arrays, if both were previously evaluated within AIDA Array Metrix. The evaluation information is stored, as mentioned above, in an AIDA Data Format (.adf) file. To be comparable, the arrays must be identical in number and order of the dots

Click the **Open** option on the **File** menu to open an existing array comparison file. Select the file you want to open from the list in the **Open AIDA Data** dialog box that appears.

The **Save** command is used to save the current comparison data. **Save As** saves the active comparison with another path or filename. **Close** closes the current images and comparison.

(See the Options Menu section for a description how to set standard parameters for the default format and search path.)

Import and **Export** allow you to exchange several evaluation data (e.g. the export of the images with their overlays). For a detailed description, see the Export and Import section below.

The **Print** command allows you to print your image and results data 'on the fly' without permanently storing the layout/printing settings (whereas the **Protocol Print** panel provides you with a complete set of printing features which includes saving the settings with the data file for reasons of reproducibility).

Printer Setup opens the standard Windows **Printer Setup** dialog box for your printer.

The **Print Preview** option shows the appearance of the currently configured printout (multiple pages) on the screen. For a detailed description of all print functions see the respective sections below.

The **Information** item, as already described in the Data Structure section above, displays a dialog box with resolution and size information of the current image file, the current evaluation type, the date of the last changes and other available evaluations.

Edit Menu

The options in the **Edit** menu are related to the current window. Their availability and specific actions depend on the active image or data window and the active overlays.

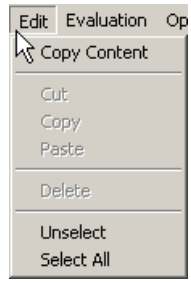


Figure 2-5 Edit Menu

Copy Content copies the content of the active data window into the clipboard using an appropriate data format. See the Export and Import section for the available options for exporting the image to the clipboard in the Windows bitmap format.

Usually **Cut**, **Copy**, **Paste** and **Delete** are used to edit overlays (e.g. text and arrows placed in the image window) or entries. Since there is nothing to edit in AIDA Array Compare data windows, these commands are disabled.

The **Select All** menu option selects all overlays present in the active image. **Unselect** cancels all selections.

Options that are not available for the currently selected item will be displayed in gray, as usual.

Evaluation Menu

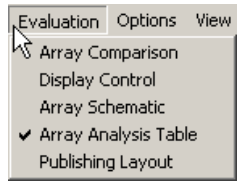


Figure 2-6 Evaluation Menu

Selecting **Array Comparison**, **Display Control**, **Array Schematic** and **Array Analysis Table** or **Publishing Layout** opens toolboxes that are explained in the toolbar section below.

Options Menu

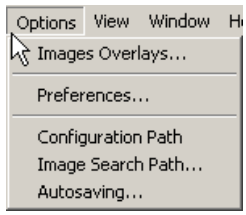


Figure 2-7 Options Menu

Various basic settings for current and future use of AIDA Array Compare can be made in the **Options** menu. Selecting an item from the menu opens a dialog box where you can specify the settings:

The **Images Overlays** option is used to change the appearance of the overlays. The options available in this dialog box depend on the image overlays defined in Array Metrix.

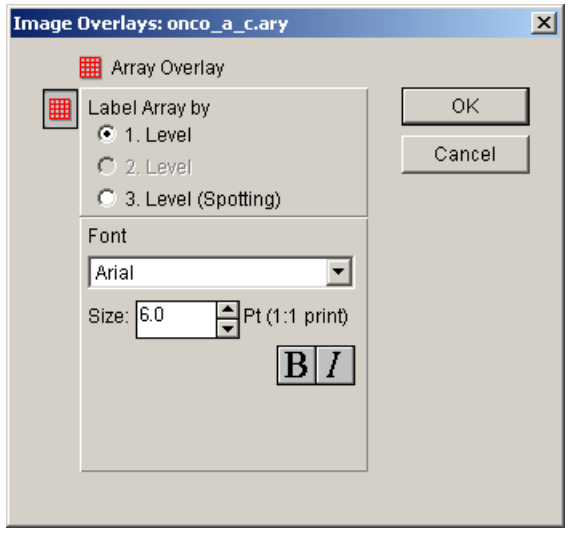


Figure 2-8 Image Overlays Dialog Box

The current settings can be saved in the **Preferences** under a user-defined name to be available for further evaluations of similar images.

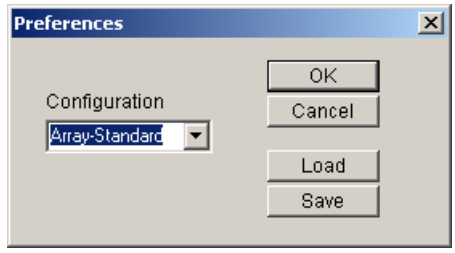


Figure 2-9 Preferences Dialog Box

Select the path for the program configuration files using the **Configuration Path** option.

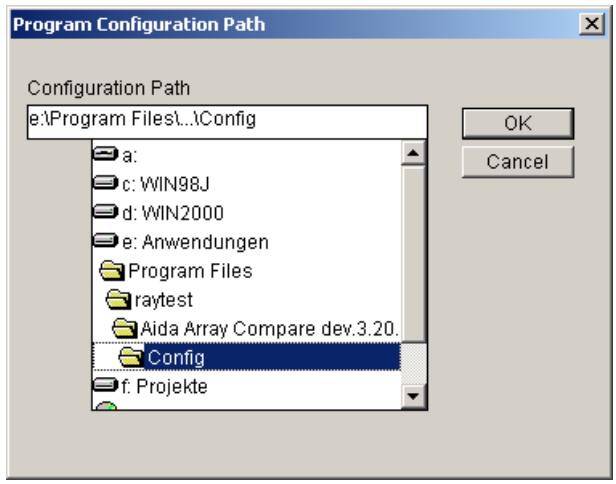


Figure 2-10 Program Configuration Path Dialog Box

In this directory all preferences files and other definable settings are stored.

Use the **Image Search Path** option to specify the default locations of image files, you can keep the images on CD while the evaluation file is stored on the hard drive, since AIDA Array Compare does not copy the raw image data into the Array Compare file. Multiple search paths can be defined.

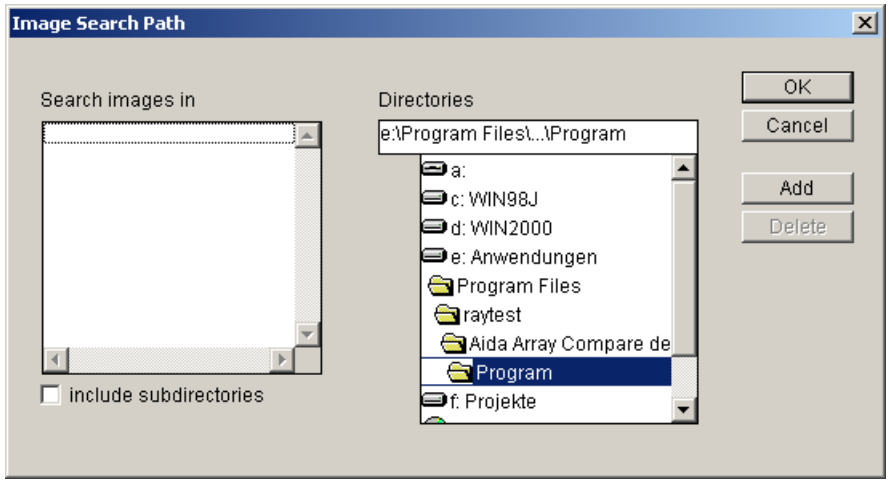


Figure 2-11 Image Search Path Dialog Box

The **Autosaving** option allows you to save your current work in the background so that there is always a recent backup when things go wrong. Enter the autosaving time interval in minutes. If you specify 0 minutes, no backup will be performed.

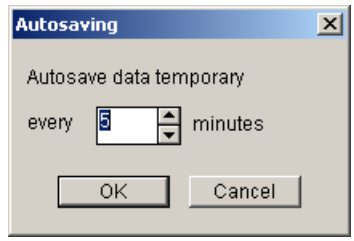


Figure 2-12 Autosaving Dialog Box

View Menu

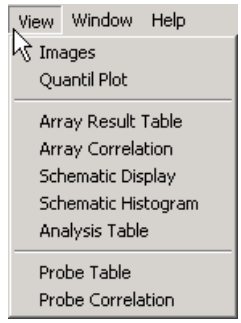


Figure 2-13 View Menu

From the **View** menu you can select different data windows available in Array Compare.

Window Menu

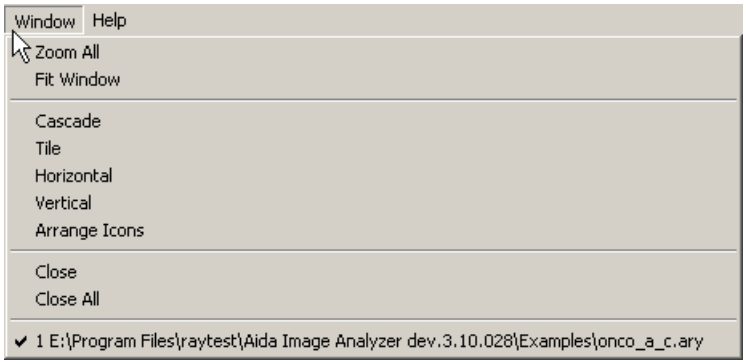


Figure 2-14 Window Menu

Select the **Zoom All** option from the **Window** menu to fit the image to the (manually changed) window frame size. To fit the frame size to the actual image size, choose **Fit Window**.

The other options in this menu are standard Windows options described in the Windows manual.

Toolbox Control Menu

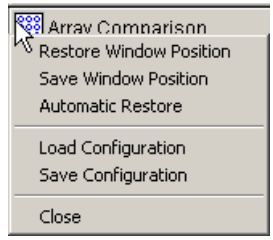


Figure 2-15 Toolbox Control Menu Example

Every toolbox has a hidden control menu attached, which you can access by clicking on the title icon in the upper left corner of the toolbox. You can use the toolbox control menu to restore (**Restore Window Position**) or save (**Save Window Position**) the position of the data windows. In addition, the configuration can be loaded from or saved to the scheme you saved using the **Preferences** option of the **Options** menu. Just click on the **Load Configuration** or **Save Configuration** menu item respectively.

Toolbar Structure

When you start AIDA Array Compare, the following toolbar is displayed:

Figure 2-16 Toolbar (on Program Startup)

On opening an array file (please follow the instructions in the Tutorial), the toolbar will change its appearance, so that all visible functions are accessible.

Figure 2-17 Toolbar (on File Opening)

Clicking an icon activates a function that in most cases can also be accessed through the menus or from the toolbox. Some functions open a toolbox, whereas others initiate a function that will be activated by one or multiple mouse clicks.

File Options (left section)

These function are also found in the **File** menu and are explained in the respective section of this manual. These functions will always effect the image and entire data set of the active window.

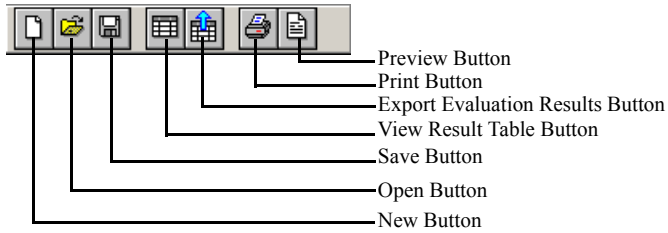


Figure 2-18 Toolbar (Left Section) Overview

The **New**, **Open**, **Safe**, **Print** and **Print Preview** buttons open the respective dialog boxes explained in the File Menu, Print and Preview. **View Result Table** opens the Array Result Table window (for details, see the respective section below). Clicking the **Export Evaluation Results** opens a dialog box for specifying a file name and path for the export file (see the Export and Import section for details)

Visual Tools (middle section)

This part of the toolbar contains all mouse-driven tools that can be activated at any time for any window, such as images, tables and graphs (some restrictions apply). Additional buttons may come up when certain toolboxes are or were open and are covered in respective toolbox sections.

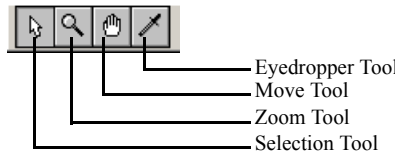


Figure 2-19 Toolbar (Middle Section) Overview

You can use the **Select** tool to pick (activate, select) dots in the windows for evaluation. The selected dot will turn magenta. To select more than one dot, press down the Ctrl key and click on the desired dots subsequently. You can also open a rectangle by clicking the position for the designated upper left corner of the rectangle, holding down the mouse button and pulling to the designated lower right corner of the desired area. All objects in the covered area will now be selected (magenta). You can deselect single objects by holding the Ctrl key and clicking them a second time.

Note that in AIDA Array Compare selecting is done simultaneously in both images with respect to the array location on each image. The same is true for all other data windows.

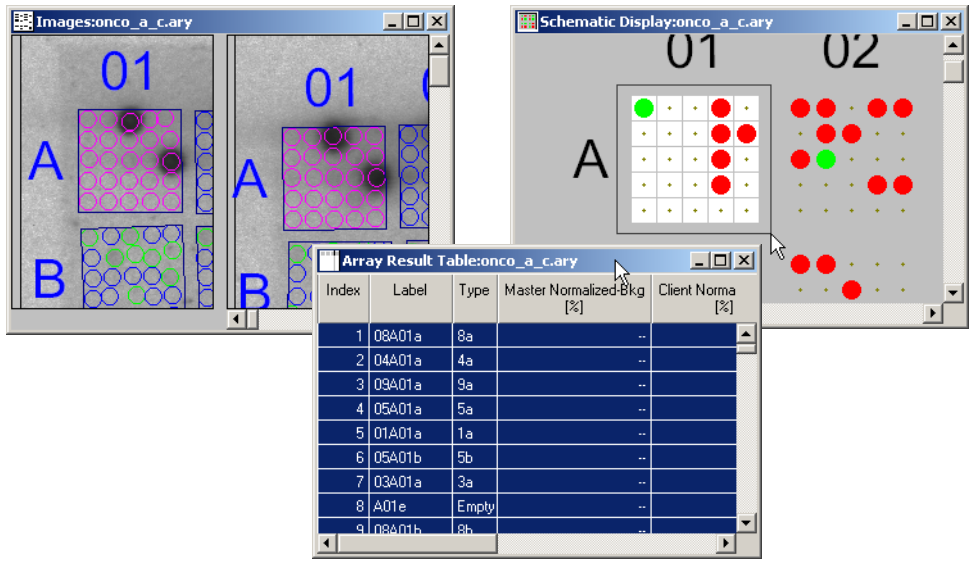


Figure 2-20 Using the Selection Tool

When the **Zoom** tool is activated, every click into one of the image windows enlarges that part and the corresponding part of the window simultaneously (centered around the mouse position) by a factor of two, leaving the window at the same size. Right-click to zoom out in the same steps.

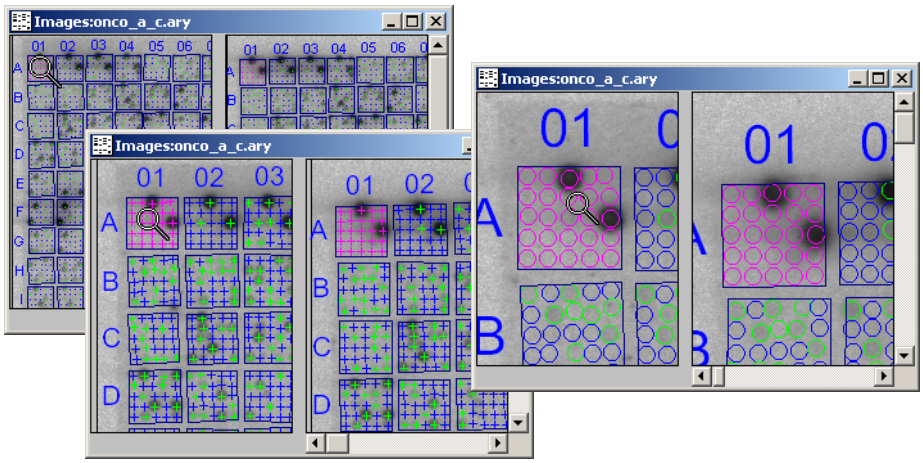


Figure 2-21 Using the Zoom Tool

The **Zoom** tool can also be used in data windows. Click in the upper left corner of the area you want to enlarge, hold down the mouse button and drag to the lower right corner. Upon release, the selected area will be displayed enlarged in the same window. The right mouse button is used to zoom out again.

The **Move** tool can be used to view parts of the window content that lie outside the window (e.g. for a zoomed image). When activated, you can click at any position in the image, hold down the mouse button and move around the window content with the pointer. The **Move** tool does not work in data windows.

Usually the **Eyedropper** tool allows you to examine pixel values at mouse position. In some graphical windows, selecting the pipette-shaped symbol enable cross hairs attached to the pointer, which runs across the curve displayed in the data window while moving the pointer horizontally with the left mouse button pressed. However, in the built-in Array Compare mode this function is similar to the selection function accessed by clicking the arrow button explained above.

Evaluation Option (right section - first half)

Clicking the **Evaluation** button, opens the **Change Evaluation Mode** dialog box.

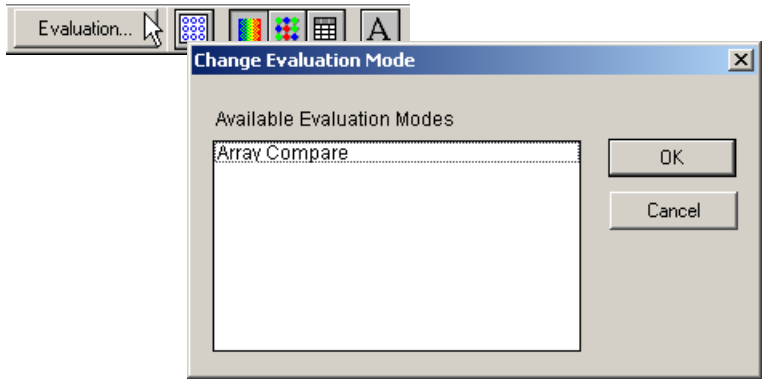


Figure 2-22 Changing Evaluation Mode

In the **Change Evaluation Mode** dialog box, you can choose an evaluation mode from the **Available Evaluation Modes list**. The content of this list reflects the installed AIDA modules. If no module is installed, only the **Array Compare** mode entry is displayed.

Toolbox functions (right section - second half)

The buttons on this part of the toolbar are used to open the various Array Compare toolboxes.For further description see the Array Compare Toolboxes section below.

Shortcut Menus

Shortcut menus are attached to all data windows and the image. They enable a comprehensive functionality without overcrowding the desktop. The menus can be accessed by right-clicking on an item in a window. The menus offer functions related to the configuration of the clicked item. Most of the time these functions are not vital for a successful evaluation, however, they will help to optimize the configuration of the output, based on personal needs. The settings in these menus can be saved using the **Preferences** command found in the **Options** menu.

Since shortcut menus are context-sensitive, they are explained together with the respective windows.

Data Windows in Array Compare

In Array Compare there are nine data windows available:

- Images
- Quantil Plot
- Array Result Table
- Array Correlation
- Schematic Display
- Schematic Histogram
- Analysis Table
- Probe Table
- Probe Correlation

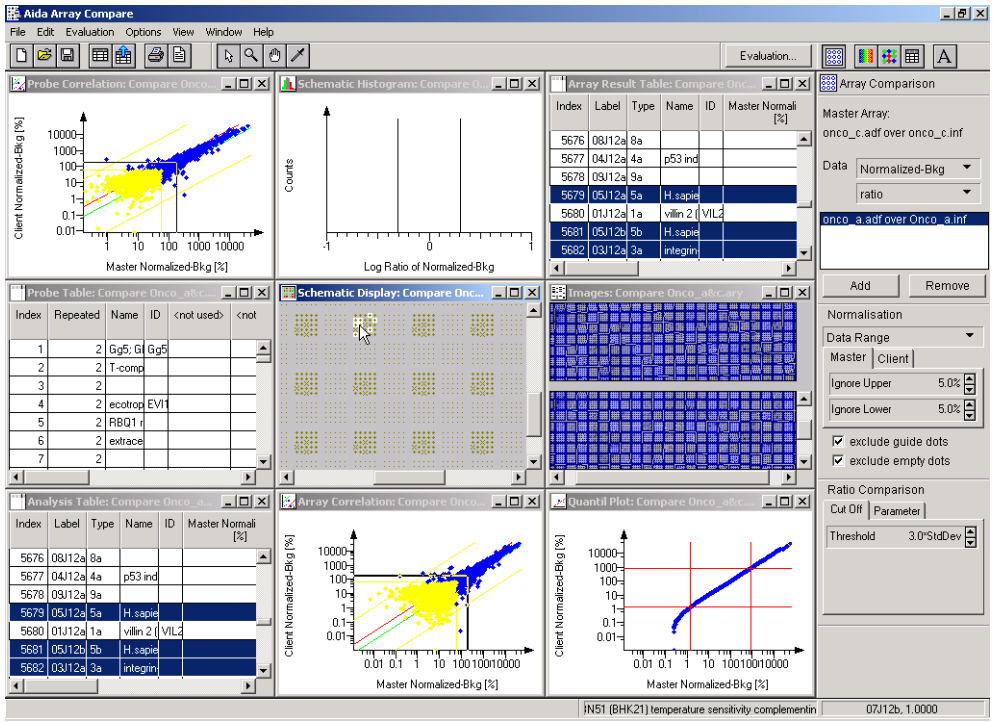


Figure 2-23 Data Windows

To access these data windows, select the appropriate entry (**Images**, **Quantil Plot**, **Array Results**, **Array Correlation**, **Schematic Display**, **Schematic Histogram**, **Analysis Table**, **Probe Table** and **Probe Correlation** respectively) from the **View** menu.

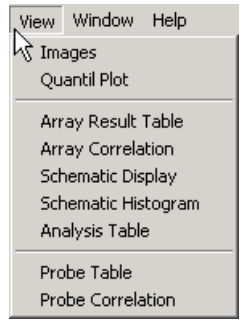


Figure 2-24 Selecting Entry From View Menu

Images Window

The **Images** window provides you with a representation of the Array Comparison data on the raw data level. It shows the image raw data together with the array overlays from which the data are calculated. Although it might not always be on top, it is always open. In AIDA Array Compare the **Images** window is bipartite, one pane for the array comparison master and one for the client.

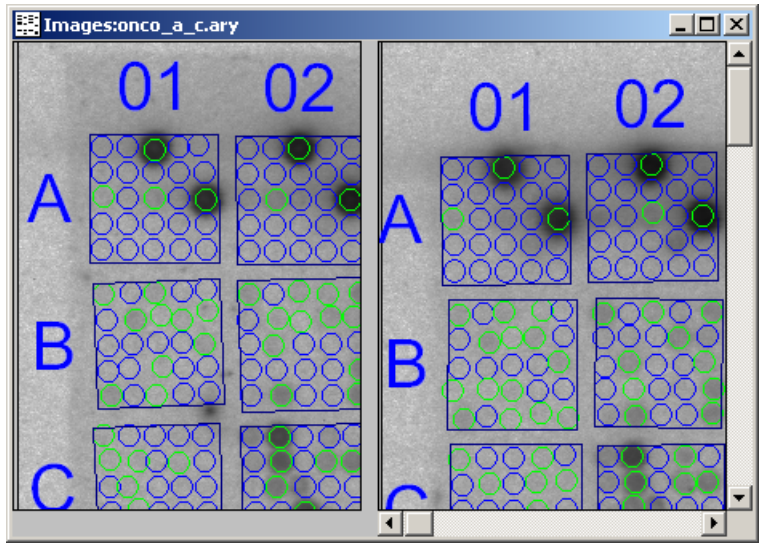
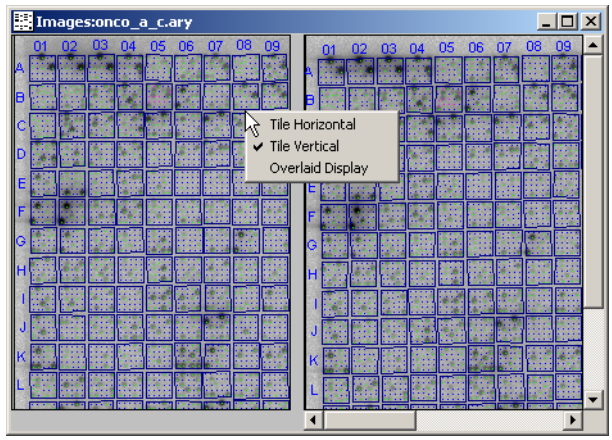


Figure 2-25 Images Window Example

To copy the content of the **Images** window to the clipboard, select the **Copy Content** option from the **Edit** menu.

Shortcut Menu of the Images Window

The shortcut menu attached to the **Images** window provides three options for arranging the two panes of the window:



Tile Horizontal and **Tile Vertical** places them side by side and on top of each other respectively. Both options use grayscale for displaying the images. The **Overlaid Display** option shows the two images superimposed on each other in false color display.

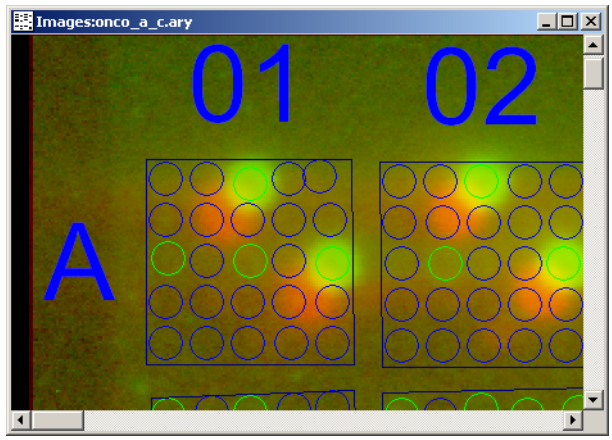


Figure 2-26 Overlaid Display Example

Quantile Plot Window

In the **Quantile Plot** window, the values of the array comparison master and the values of the client are each sorted by quantity and then plotted against each other. Using this data view you can easily check for saturation issues occurring in slide evaluations.

Generally, the Quantile view allows conclusions on the normalization quality.

To display the Quantile Plot window, select **Quantile Plot** from the View menu.

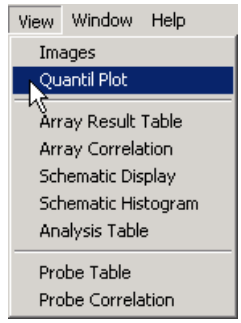


Figure 2-27 Selecting Quantile Plot

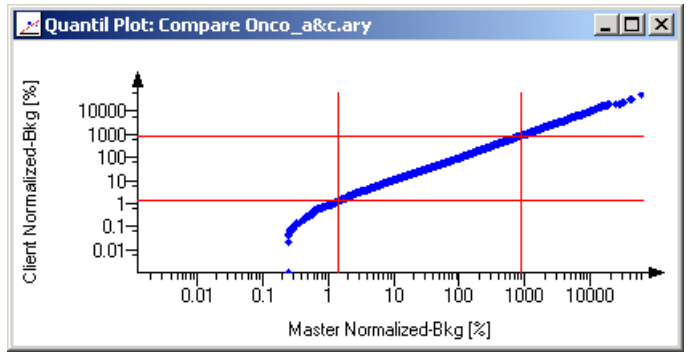


Figure 2-28 Quantile Plot Windows Example

Array Result Table Window

This window shows the results of the array comparison in tabular form. To display the **Array Result Table** window, select the **Array Results** option from the **View** menu.

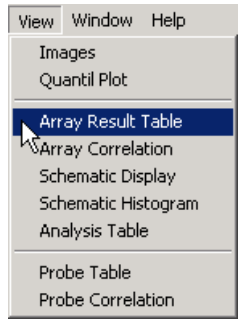


Figure 2-29 Selecting Array Results

Array Result Table:onco_a_c.ary

Index	Label	Type	Master Normalized-Bkg [%]	Client Normalized-Bkg [%]	Ratio Normalized-Bkg	Expr
1	08A01a	8a	--	--	0.0	
2	04A01a	4a	--	--	1.6	
3	09A01a	9a	--	--	1.1	
4	05A01a	5a	--	--	2.3	
5	01A01a	1a	--	--	1.1	
6	05A01b	5b	--	--	1.0	
7	03A01a	3a	--	--	1.0	
8	A01e	Empty	--	--	0.9	
9	08A01b	8b	--	--	2.9	
10	07A01a	7a	--	--	5.6	
11	10A01a	10a	--	--	1.0	
12	07A01b	7b	--	--	1.0	
13	A01g	Guide	--	--	0.6	
14	06A01a	6a	--	--	2.4	
15	09A01b	9b	--	--	1.2	

Figure 2-30 Array Result Table Example

Data Description

The possible columns of the **Array Result Table** are the following:

Table 2-1 Columns in Array Result Table

Columns	Short Description
Index	Running number
Label	Label of each spot in the array
Type	Type of spot (empty, guide, reference, background)
Master *data*	Chosen comparison data of master array
Client *data*	Chosen comparison data of client array
Master Quality	Quality flag of master array dots (read from the **Quality** column of the master array's Array Metrix **Array Result Table**) - indicates artefacts automatically or manually identified
Client Quality	Quality flag of client array dots (read from the **Quality** column of the client array's Array Metrix **Array Result Table**) - indicates artefacts automatically or manually identified
Result	Result of chosen comparison data
Expression	Value of the Result column encoded according to the following scheme: **On**: result value of master within background range *and* client's result value above background **Off**: result value of master above background and client's result value within background range ***Ratio***: ratio value > 1 ***-1/Ratio***: ratio value < 1
Row Index	Global grid position of a dot (row component; potential reference column for name table import)
Column Index	Global grid position of a dot (column component; potential reference column for name table import)
Row 1	Subgrid row position of the dot (1. level; potential reference column for name table import)
Row 2	Subgrid row position of the dot (2. level; potential reference column for name table import)
Row 3	Subgrid row position of the dot (spot matrix level; potential reference column for name table import)

where *data* denotes one of the following, selectable from the **Data** drop-down menu of the **Array Comparison** toolbox

Table 2-1 Columns in Array Result Table

Columns	Short Description
Column 1	Subgrid column position of the dot (1. level; potential reference column for name table import)
Column 2	Subgrid column position of the dot (2. level; potential reference column for name table import)
Column 3	Subgrid column position of the dot (spot matrix level; potential reference column for name table import)
Label 1	Label first level
Label 2	Label second level
Label 3	Label third level
Row Label 1	Subgrid label of the dot (row component, 1. level)
Row Label 2	Subgrid label of the dot (row component, 2. level)
Row Label 3	Subgrid label of the dot (row component, spot matrix level)
Column Label 1	Subgrid label of the dot (column component, 1. level)
Column Label 2	Subgrid label of the dot (column component, 2. level)
Column Label 3	Subgrid label of the dot (column component, spot matrix level)
Name	Name of a dotted sample imported from name table (column name can be adjusted and cells can be linked to external database by the user via column shortcut menu command)
ID	ID-number of a dotted sample imported from name table (column name can be adjusted and cells can be linked to external database by the user via column shortcut menu command)
<unused>	User-definable columns for name table import purpose (column name can be adjusted and cells can be linked to external database by the user via column shortcut menu command)

where *data* denotes one of the following, selectable from the **Data** drop-down menu of the **Array Comparison** toolbox

Table 2-2 Data Types

Data Type	Short Explanation
Integral [ ]	Integrated raw intensity of a spot
Integral-Bkg [ ]	Integral background corrected
Integral/Area [ ]	Integral divided by area of a spot

Table 2-2 Data Types

Data Type	Short Explanation
Integral/Area-Bkg [ ]	Integral per area background corrected
Normalized [%]	Integral divided by the reference value
Normalized-Bkg [%]	Integral minus Background divided by reference

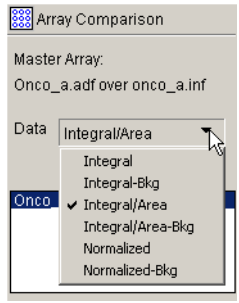


Figure 2-31 Selecting Data Type Using Data Drop-Down Menu

Result depends on the mathematical operation selected from the second **Data** drop-down menu of the **Array Comparison** toolbox (see the respective section below), which includes **Ratio** *data* and **Difference** *data*.

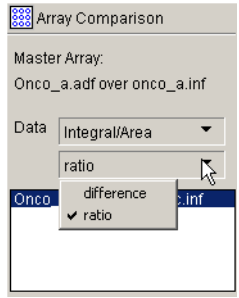


Figure 2-32 Selecting Mathematical Data Operation

If one or several dots are selected in the **Images** window, the corresponding rows in the **Array Result Table** are also highlighted, and vice versa.

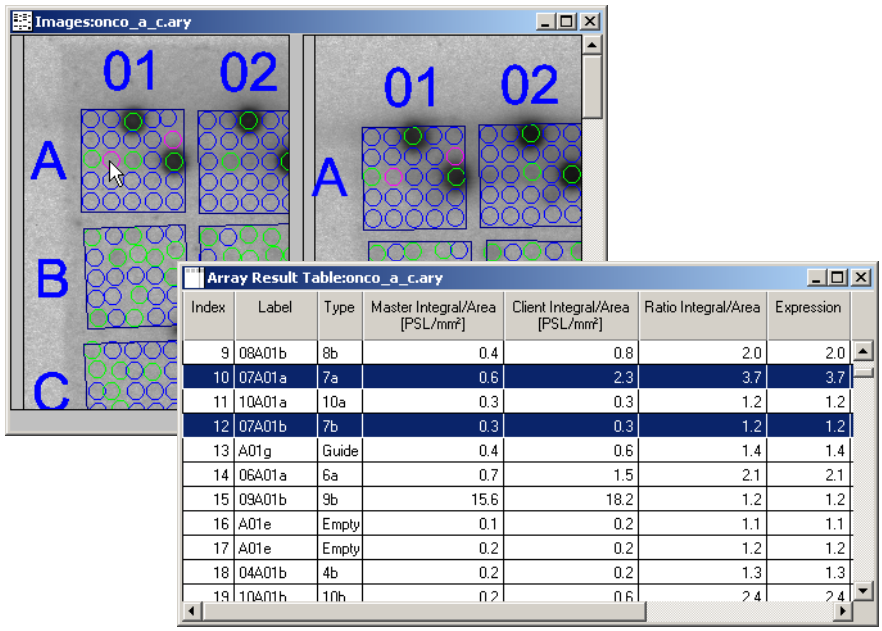

Images:onco_a_c.ary
01
02
A
B
C
01
02
A
Array Result Table:onco_a_c.ary
Index | Label | Type | Master Integral/Area [PSL/mm²] | Client Integral/Area [PSL/mm²] | Ratio Integral/Area | Expression
9 | 08A01b | 8b | 0.4 | 0.8 | 2.0 | 2.0
10 | 07A01a | 7a | 0.6 | 2.3 | 3.7 | 3.7
11 | 10A01a | 10a | 0.3 | 0.3 | 1.2 | 1.2
12 | 07A01b | 7b | 0.3 | 0.3 | 1.2 | 1.2
13 | A01g | Guide | 0.4 | 0.6 | 1.4 | 1.4
14 | 06A01a | 6a | 0.7 | 1.5 | 2.1 | 2.1
15 | 09A01b | 9b | 15.6 | 18.2 | 1.2 | 1.2
16 | A01e | Empty | 0.1 | 0.2 | 1.1 | 1.1
17 | A01e | Empty | 0.2 | 0.2 | 1.2 | 1.2
18 | 04A01b | 4b | 0.2 | 0.2 | 1.3 | 1.3

Figure 2-33 Interlinkage between Images and Array Result Table Window

You can select the number and type of columns you want to display using the shortcut menu of any column in the window (see below).

If you want to change the column order, click the heading of the column to be moved, hold down the mouse button and drag to the desired position in the table. Release the mouse button.

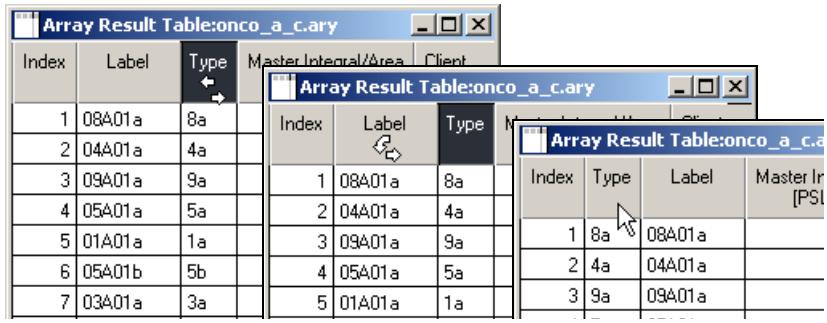

Array Result Table:onco_a_c.ary
Index | Label | Type
1 | 08A01a | 8a
2 | 04A01a | 4a
3 | 09A01a | 9a
4 | 05A01a | 5a
5 | 01A01a | 1a
6 | 05A01b | 5b
7 | 03A01a | 3a
Array Result Table:onco_a_c.ary
Index | Label | Type
1 | 08A01a | 8a
2 | 04A01a | 4a
3 | 09A01a | 9a
4 | 05A01a | 5a
5 | 01A01a | 1a
Array Result Table:onco_a_c.a
Index | Type | Label
1 | 8a | 08A01a
2 | 4a | 04A01a
3 | 9a | 09A01a

Figure 2-34 Changing the Column Order

To change the column width, position the mouse pointer at the border of the column whose width you want to change then click and drag the border in the appropriate direction. Release the mouse button.

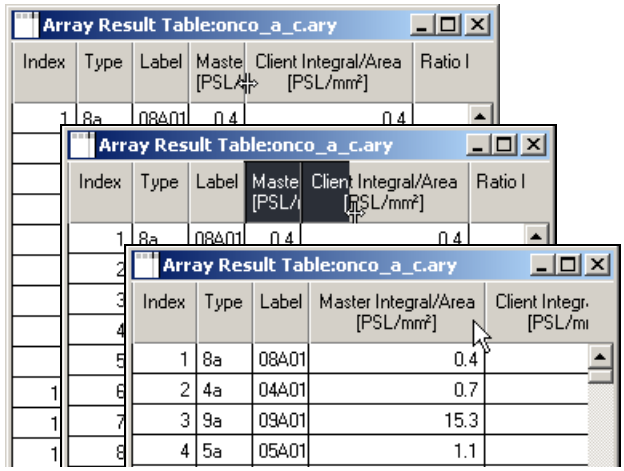


Figure 2-35 Changing Column Width

Shortcut Menu of the Array Result Table Window

Each column in the **Array Result Table** has a shortcut menu attached.

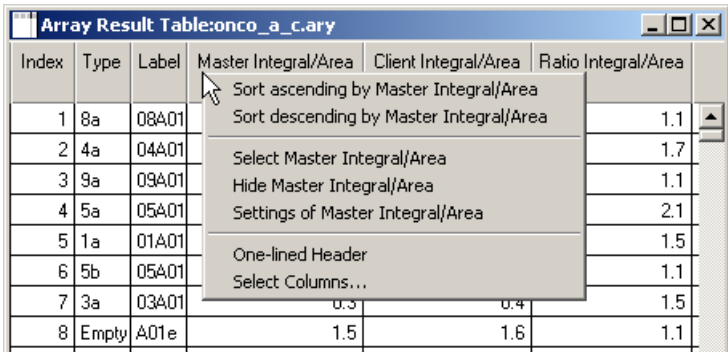


Figure 2-36 Shortcut Menu (Array Result Table)

The table can be sorted by any value column in ascending order (A to Z or zero to 9) or descending order (Z to A or 9 to zero). Just choose the appropriate menu item (**Sort ascending by**/**Sort descending by**)

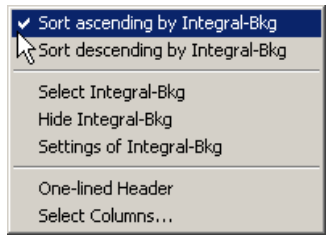


Figure 2-37 Sorting Table

This is especially of interest for finding highest intensity values and for identifying the highest absolute or relative deviations in double dotted samples for quality control.

The **Select**/**Unselect** options of the shortcut menu are used to select/unselect a column to export. The column heading of selected columns are highlighted.

To hide a column, select the **Hide** option from its shortcut menu. You can recover hidden columns using the **Select Columns** option (see below).

To specify the decimal digits for the value output in a column, choose **Setting of** from the shortcut menu and enter the value for the decimal digits in the dialog box that appears.

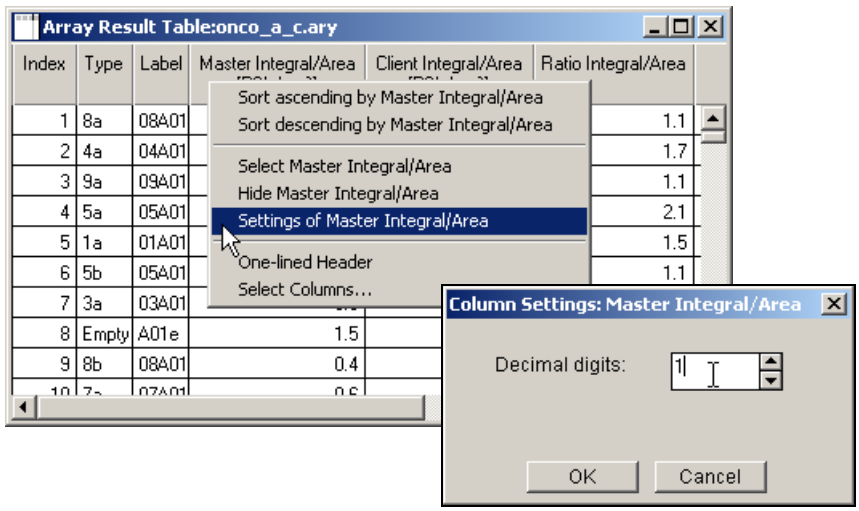


Figure 2-38 Specifying Decimal Digits Display

Note that the behavior of the **Settings of** option depends on the column you perform the right-click: Columns whose cell contents is usually provided via name table import, i.e. the **Name** and the **ID** column as well as the two user-definable columns with the default name **<unused>**, have **Column Settings** dialog boxes totally different from the ones provided by measurement value output columns.

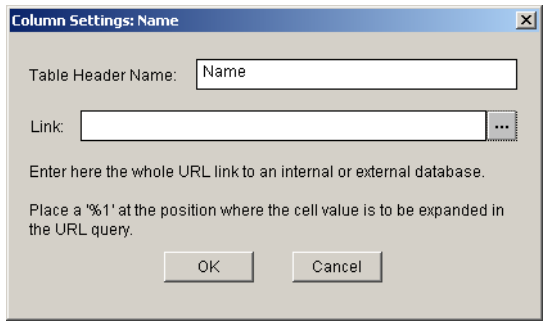

Column Settings: Name
Table Header Name:
Name
Link:
Enter here the whole URL link to an internal or external database.
Place a '%1' at the position where the cell value is to be expanded in the URL query.
OK
Cancel

Figure 2-39 Column Settings Dialog Box (Name Column)

Instead of decimal digit specifications, you can edit the column's default name and enter a URL (Uniform Resource Locator) to link the column's cells to internal or external databases (see the Importing Name Tables section for details)

Use the **One-lined Header**/**Two-lined Header** option to reduce or expand the column heading by one line (the second line indicates the units of measurement).

To select the columns that should be displayed in the table, choose the **Select Columns** option.

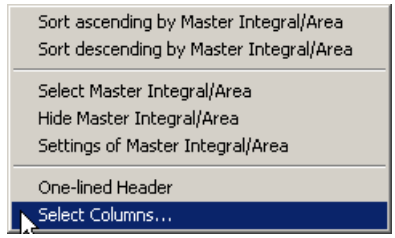


Figure 2-40 Select Columns Command of the Shortcut Menu

A dialog box appears displaying two lists (titled: **Show Columns**/**Hide Columns**). Select the columns you want to show or hide and click the appropriate arrow symbol to transfer the items between the two lists. To unselect a particular item, just click it again.

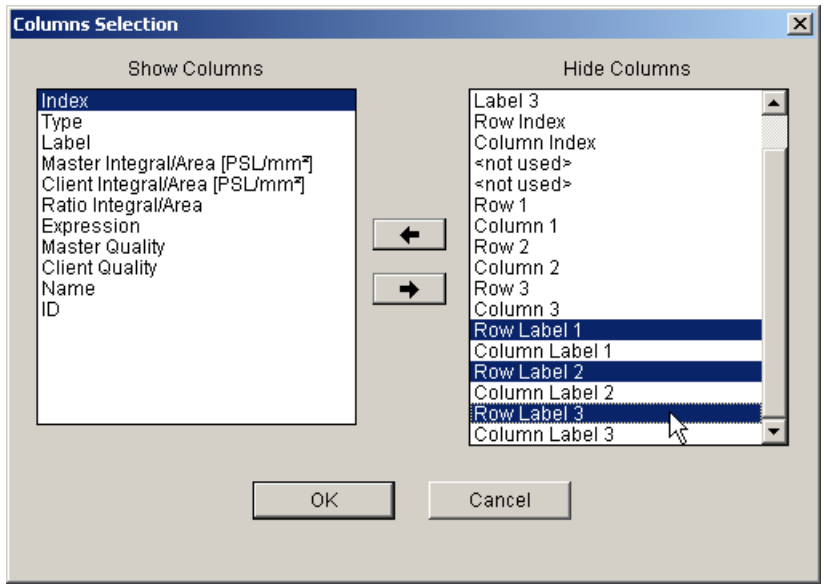


Figure 2-41 Columns Selection Dialog Box

Array Correlation Window

In the **Array Correlation** window the data of the master array are plotted on the x-axis against the data of the client array on the y-axis. The mean of the Log ratios of the two arrays (gray line) and the data/mean thresholds (color-coded lines) configurable via the **Array Schematic** toolbox are represented (see the Reviewing Array Comparison Data section for a more detailed description).

To open the **Array Correlation** window:

Select the **Array Correlation** option from the **View** menu.

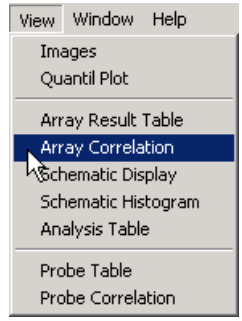


Figure 2-42 Opening Array Correlation Window

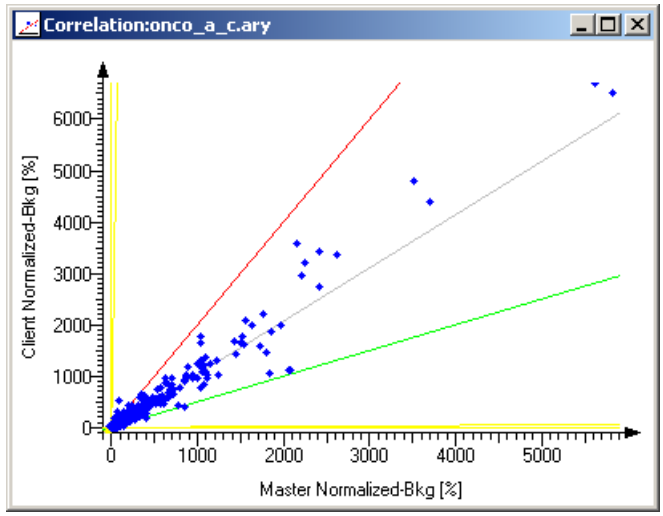


Figure 2-43 Array Correlation Window Example

For a more detailed view of the plot, the **Array Correlation** window provides a zoom function:

To zoom the correlation plot:

1 Click the Selection tool on the main toolbar.

Figure 2-44 Selection Tool on Main Toolbar

2 Drag one of the axis with left mouse button pressed.

3 Release the mouse button.

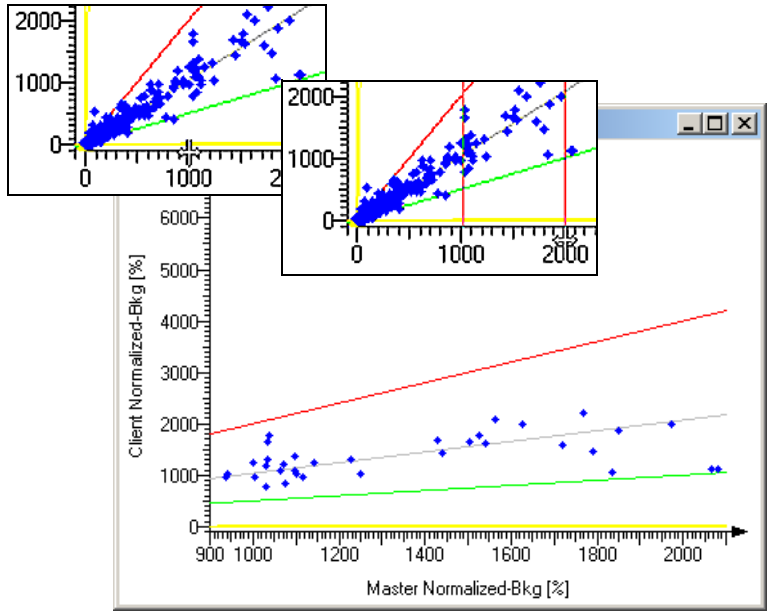


Figure 2-45 Zooming Array Correlation Plot

Alternative procedure:

1 Click the Zoom tool on the main toolbar.

Figure 2-46 Zoom Tool on the Main Toolbar

2 Draw a rectangle around the area of the plot you want to see in more detail.

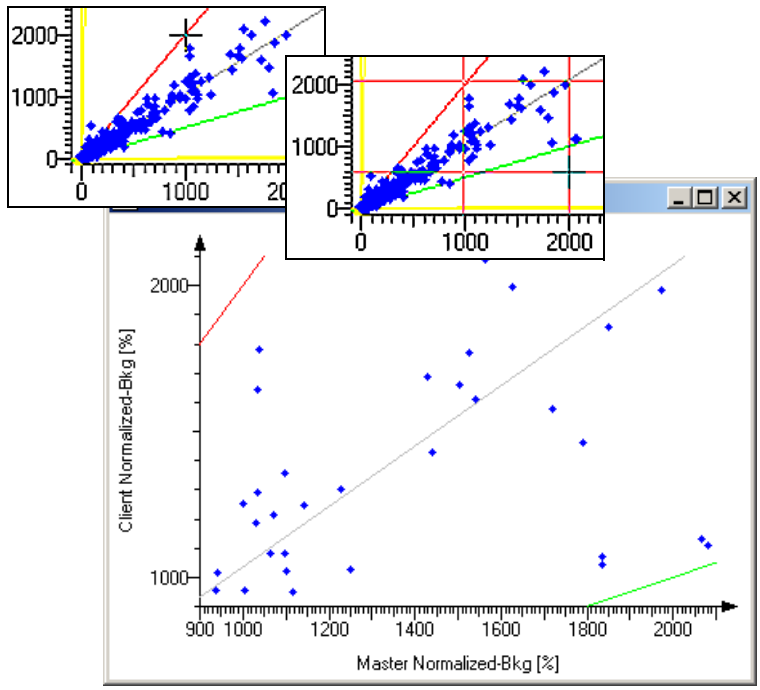


Figure 2-47 Zooming Array Correlation Plot (Alternative Procedure)

To review the data value of dot pairs (master-client) shown in the **Array Correlation** window, click one of the respective dots. The coordinates of the dot pair are highlighted, together with all repetitions of the respective dots.

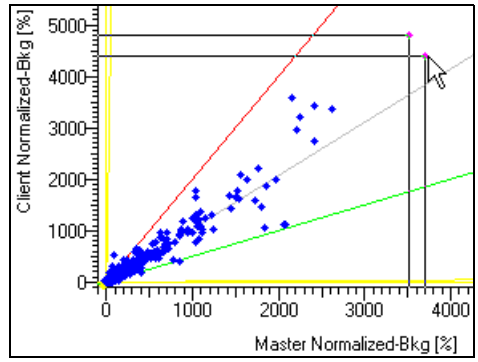


Figure 2-48 Reviewing Array Correlation Plot Values

Shortcut Menu of the Array Correlation Window

The shortcut menu of the **Array Correlation** window provides two options: **Scales** and **Grid**.

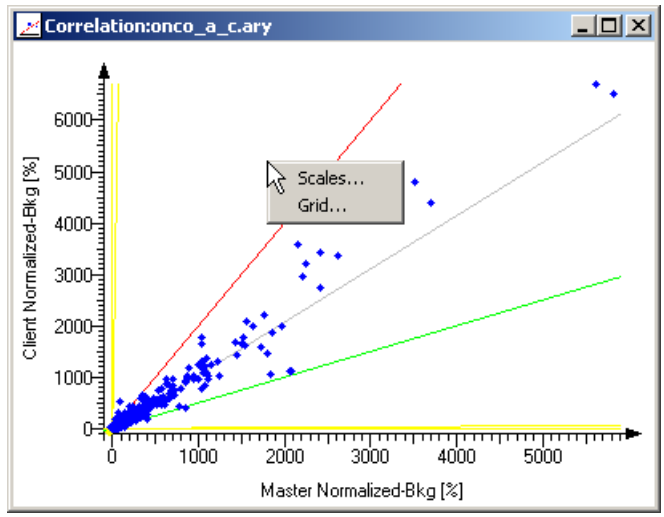


Figure 2-49 Array Correlation Window (Shortcut Menu)

The **Scales** option allows you to configure the graphical output. After selecting the **Scales** option, the **Date Scaling** dialog box opens.

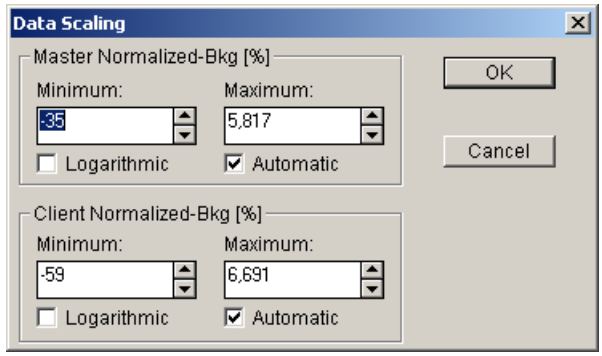


Figure 2-50 Data Scaling Dialog Box

Here the **Minimum** and **Maximum** value of the x-axis (**Master Normalized-Bkg [%]**) and y-axis (**Client Normalized-Bkg [%**]) can be selected. In addition, you can enable logarithmic representation and automatic scaling of either axis separately by checking the respective option (**Logarithmic** and **Automatic**) below the **Minimum** and **Maximum** spin boxes.

Choosing the **Grid** option opens the **Grid** dialog box, which allows you to lay grids over both axis that are an extension of either the big or the small ticks on the axes.

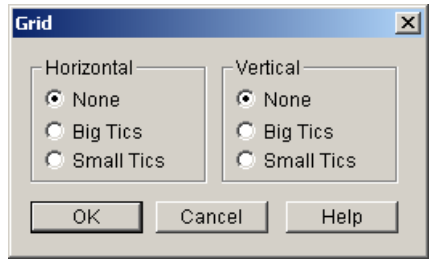


Figure 2-51 Grid Dialog Box

Schematic Display Window

To open the **Schematic Display** window, choose **Schematic Display** from the **View** menu:

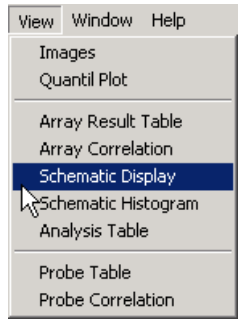


Figure 2-52 Choosing the Schematic Display Command from the View Menu

The **Schematic Display** window provides you with a schematic drawing of the array. All data within a specific data range are represented by spots of identical color.

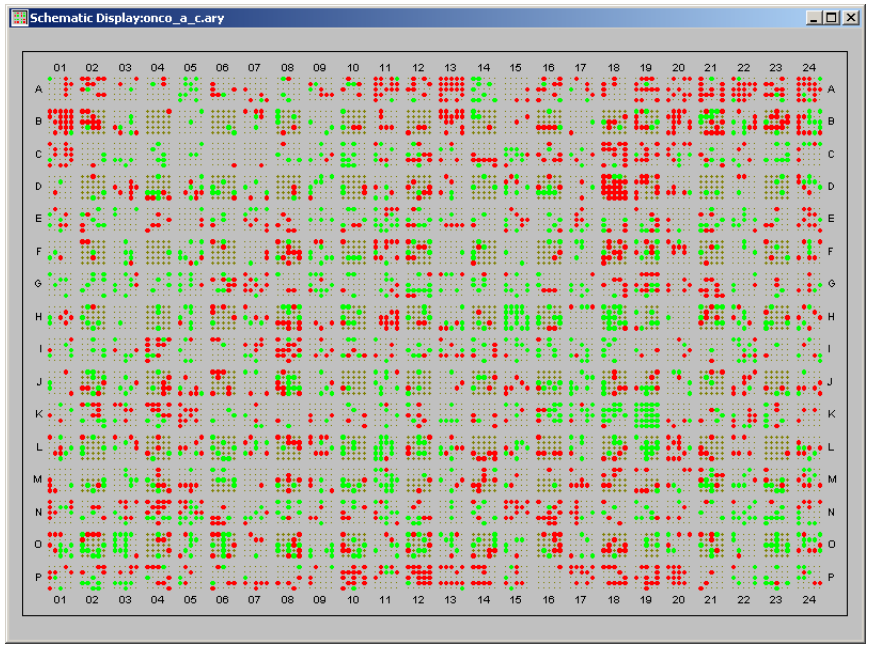


Figure 2-53 Schematic Display Window Example

Alternatively, the deviation of the spots to the mean value of data of interest can be coded by color.

The display options for **Schematic Display** window are set using the **Array Schematic** toolbox (see the respective section below for details of how to use the **Array Schematic** toolbox).

Because of the permanent linkage between all data windows, you can use the **Schematic Display** window to easily navigate through the array on the **Images** window in order to select spots or spot groups.

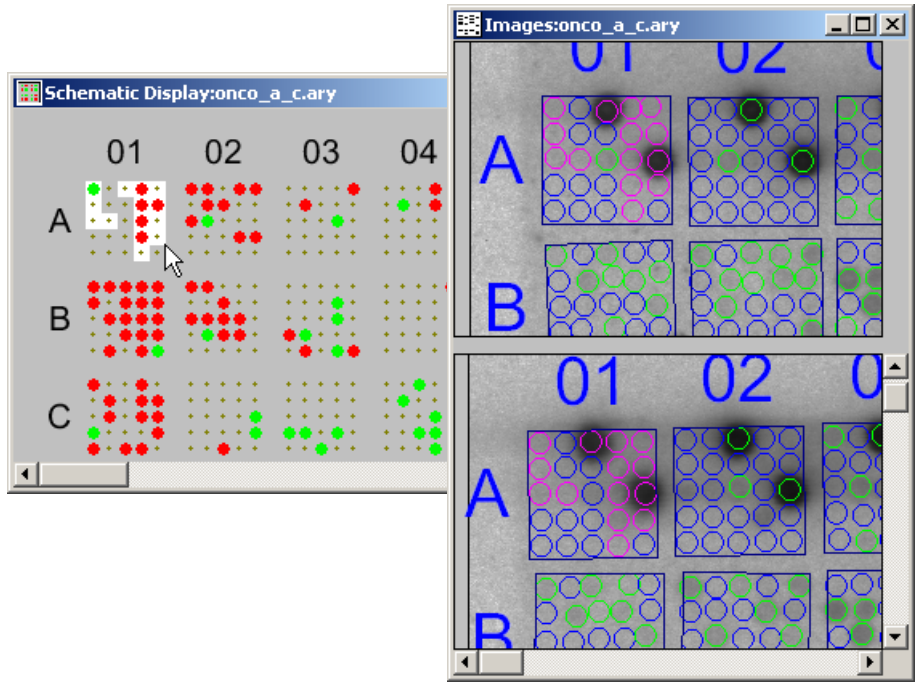


Figure 2-54 Interlinked Windows

Schematic Histogram Window

This data window shows a graphical representation of the distribution of values for a given data type (selected via the controls of the **Schematic Display** panel on the **Array Schematic** toolbox) as displayed in the **Schematic Display** window.

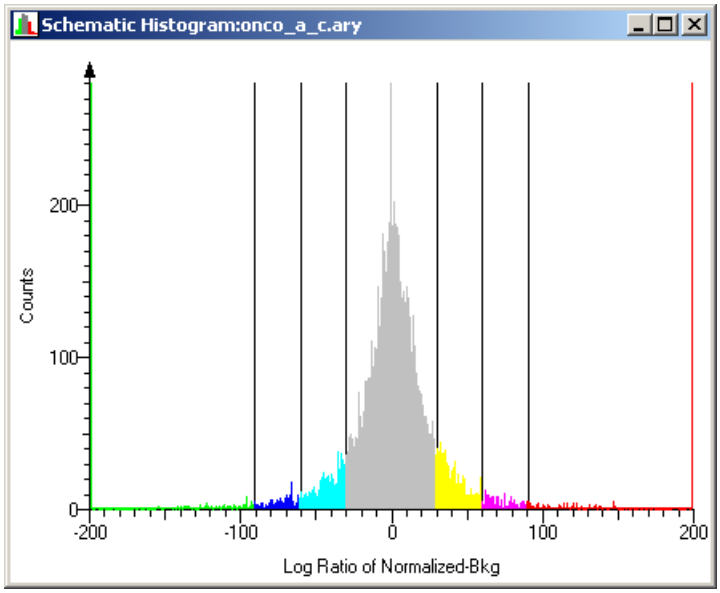


Figure 2-55 Schematic Histogram Window Example

Using the **Schematic Display** panel on the **Array Schematic** toolbox, you can configure the **Schematic Histogram** (and the **Schematic Display**) window to display certain ranges of data values in different colors.

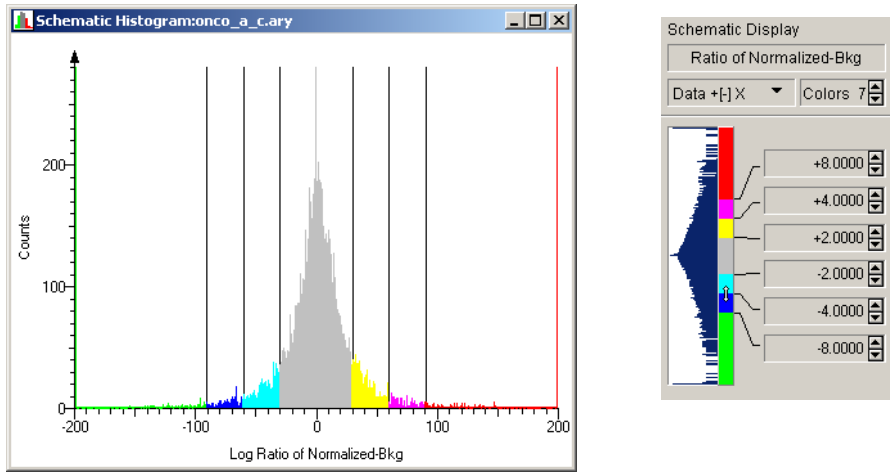


Figure 2-56 Defining Color Coding Scheme

For a more detailed view of the histogram, the **Schematic Histogram** window provides a zoom function similar to the one provided by the **Array Correlation** window (see the Array Correlation Window section for a detailed description of how to use the zoom function).

Shortcut Menu of the Schematic Histogram Window

The shortcut menu of the **Schematic Histogram** window provides one option: **Scales**.

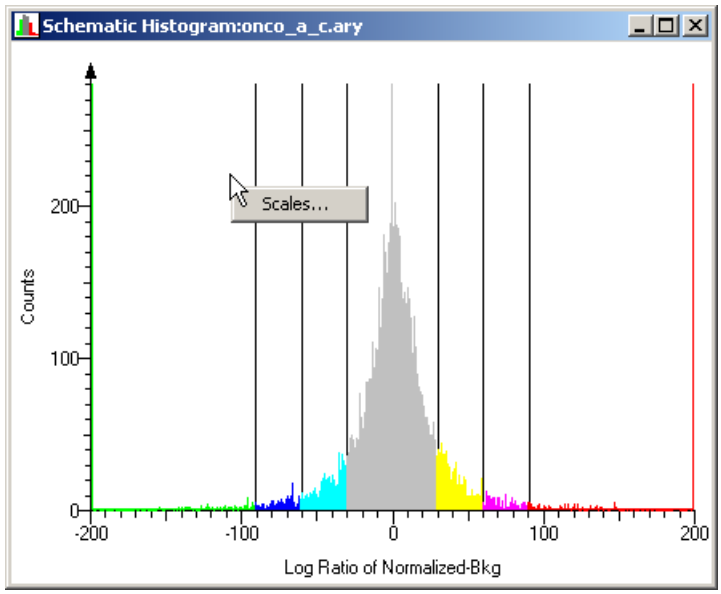


Figure 2-57 Schematic Histogram Window (Shortcut Menu)

The **Scales** option allows you to configure the graphical output. After selecting the **Scales** option, the **Date Scaling** dialog box opens.

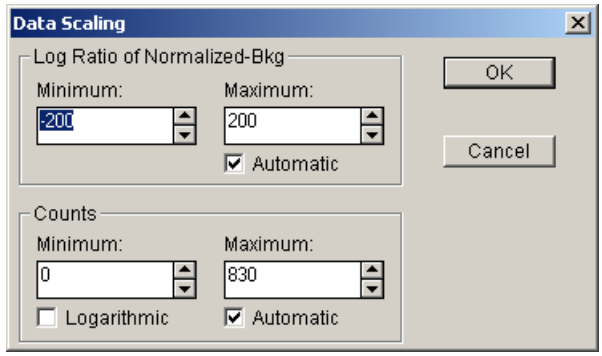


Figure 2-58 Data Scaling Dialog Box

Here the **Minimum** and **Maximum** value of the x-axis (**Log Ratio of Normalized-Bkg [%]**) and y-axis (**Counts**) can be selected. In addition, you can enable automatic scaling of either axis separately and logarithmic representation for the y-axis only by checking the respective option (**Logarithmic** and **Automatic**) below the **Minimum** and **Maximum** spin boxes.

Analysis Table Window

In contrast to the **Array Result Table** window, which lists the analysis results for the entire array, the **Analysis Table** window is used to display analysis results for a subset of the array.

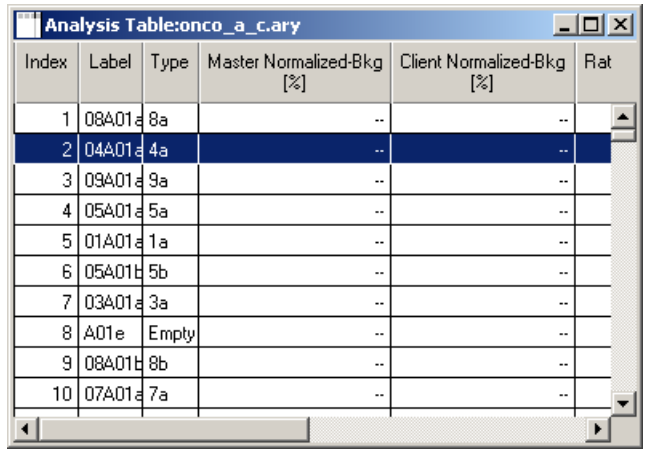


Figure 2-59 Analysis Table Window Example

You can define those subsets in two different ways: manually or by filtering using user-defined rules (for details, see the Array Analysis Table Toolbox section of this manual).

The **Analysis Table** window provides the same set of display controls as the **Array Result Table** window, so please refer to the respective description in the Array Result Table Window section for further details.

Probe Table Window

The **Probe Table** window lists the ratios of the averaged master and the averaged client values of repeated spots in a probe. Only “good” repetitions are used for the calculation of the averaged values.
To display the **Probe Table** window, select **Probe Table** from the **View** menu.

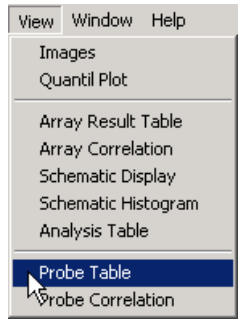
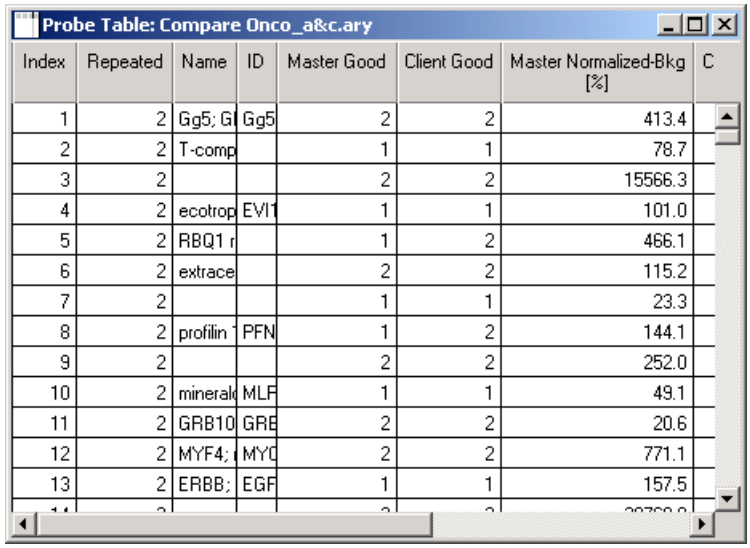


Figure 2-60 Selecting Probe Table View



Index	Repeated	Name	ID	Master Good	Client Good	Master Normalized-Bkg [%]	C
1	2	Gg5; Gl	Gg5	2	2	413.4	
2	2	T-comp		1	1	78.7	
3	2			2	2	15566.3	
4	2	ecotrop	EVI1	1	1	101.0	
5	2	RBQ1 r		1	2	466.1	
6	2	extrace		2	2	115.2	
7	2			1	1	23.3	
8	2	profilin	PFN	1	2	144.1	
9	2			2	2	252.0	
10	2	mineral	MLF	1	1	49.1	
11	2	GRB10	GRB	2	2	20.6	
12	2	MYF4;	MYO	2	2	771.1	
13	2	ERBB;	EGF	1	1	157.5	

Figure 2-61 Probe Table Window Example

Data Description

The possible columns of the **Probe Table** are the following:

Table 2-3 Probe Table Columns

Column	Short Description
Index	Running number
Repeated	Number of repetitions of the probe in the array, as determined by the spotting pattern
Master Good	Number of repetitions in the master array *not* flagged for “Bad quality”
Client Good	Number of repetitions in the client array *not* flagged for “Bad quality”
Master *data*	Selected master array probe value (average of the “Good” dots’ values)
Client *data*	Selected client array probe value (average of the “Good” dots’ values)
Ratio *data*	Ratio between Master *data* and Client *data*
Expression	Ratio *data* value encoded according to the following scheme: **On**: value of master within background range *and* client’s value above background **Off**: value of master above background and client’s value within background range ***Ratio***: ratio value > 1 ***-1/Ratio***: ratio value < 1
Name	Name of the probe, corresponds to the **Name** column of the **Array Result Table**
ID	ID of the probe, corresponds to the **ID** column of the **Array Result Table**
<not used>	User-definable columns, corresponds to the respective columns of the **Array Result Table**

where *data* denotes one of the following, selectable from the **Data** drop-down menu of the **Array Comparison** toolbox

Table 2-4 Data Types

Data Type	Short Explanation
Integral [ ]	Integrated raw intensity of a spot
Integral-Bkg [ ]	Integral background corrected
Integral/Area [ ]	Integral divided by area of a spot
Integral/Area-Bkg [ ]	Integral per area background corrected
Normalized [%]	Integral divided by the reference value
Normalized-Bkg [%]	Integral minus Background divided by reference

Probe Correlation Window

In the **Probe Correlation** window the probe data of the master array are plotted on the x-axis against the probe data of the client array on the y-axis. The mean of the Log ratios of the two arrays (gray line) and the data/mean thresholds (color-coded lines) configurable via the **Array Schematic** toolbox are represented (see the Reviewing Array Comparison Data section for a more detailed description).

To open the **Probe Correlation** window:

Select the **Probe Correlation** option from the **View** menu.

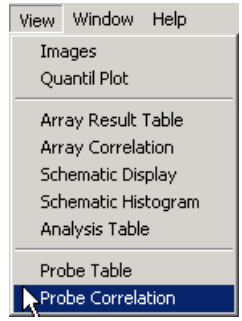


Figure 2-62 Selecting Probe Correlation View

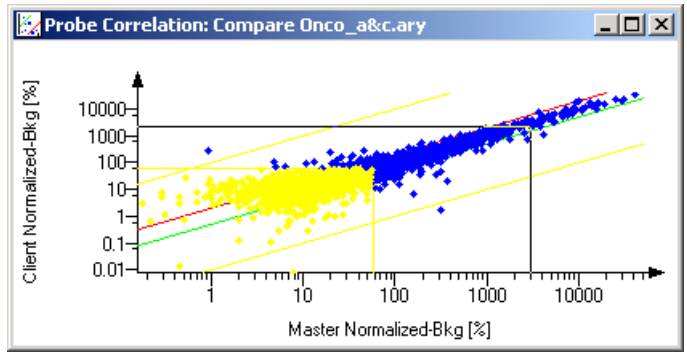


Figure 2-63 Probe Correlation Window Example

The **Probe Correlation** window provides the same set of display controls as the **Array Correlation** window, so please refer to the respective description in the Array Correlation Window section for further details.

Toolboxes of Array Compare

In Array Compare there are five toolboxes available:

- Array Comparison
- Display Control
- Array Schematic
- Array Analysis Table
- Publishing Layout

You can access these five toolboxes via the **Evaluation** menu or by clicking the appropriate button on the toolbar.

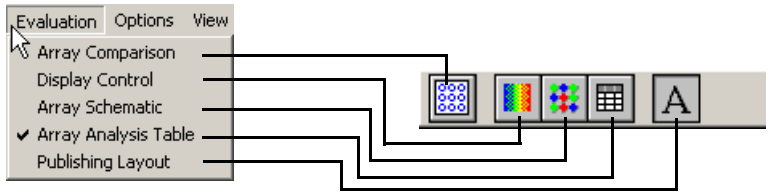


Figure 2-64 Evaluation Menu-Toolbar Correspondences

Array Comparison Toolbox

The **Array Comparison** toolbox allows you to control the comparison of arrays.

To display the **Array Comparison** toolbox:

On the main menu, click **Evaluation**, and then click **Array Comparison**. Alternatively you can select the **Array Comparison** button on the toolbar.

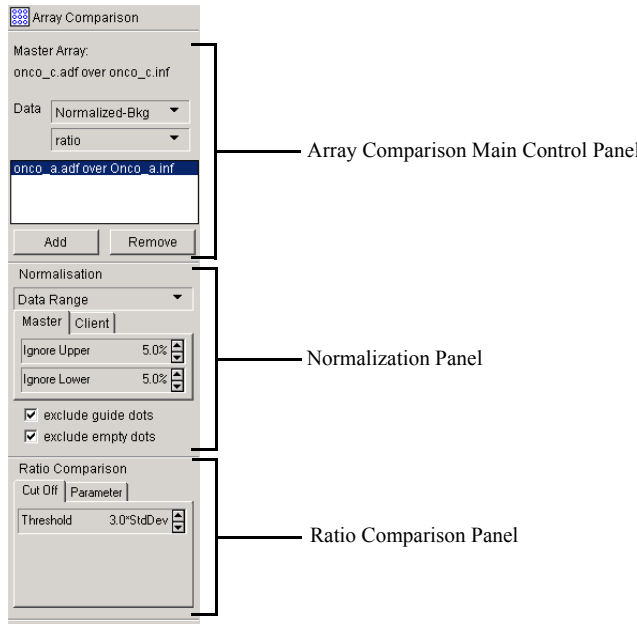


Figure 2-65 Array Comparison Toolbox Overview

Array Comparison Main Control Panel

Using the Array Comparison control panel, you can specify the basic settings for comparing arrays.

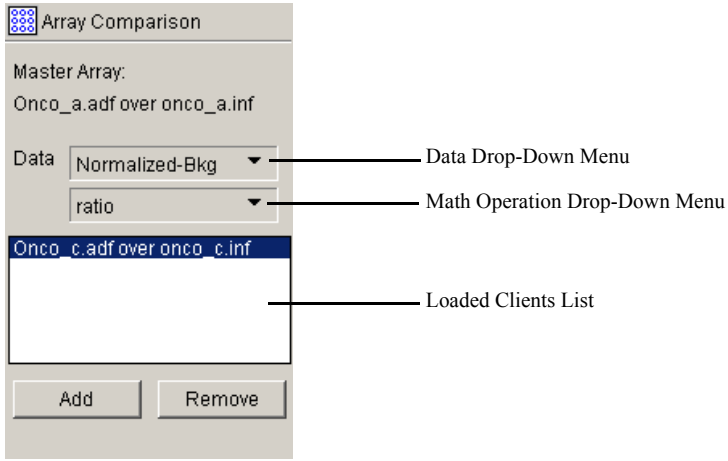


Figure 2-66 Array Comparison Main Control Panel Overview

The upper part of the control panel displays the name of the master array file.

Client array are loaded to the master for comparison by clicking the **Add** button on the bottom of the control panel (alternatively you can select **Array Evaluation** from the **Import** cascading menu of the **File** menu). Although the comparison is done only between two arrays at a time, up to four client arrays can be applied to the master array. Scrolling through the client array list gives an impression of the behavior of a single dot position over the different arrays.

With the **Remove** button, a selected client array is removed from the list.

The **Data** drop-down menu allows you to select the type of data to be compared.

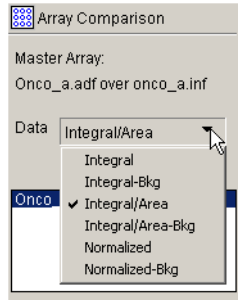


Figure 2-67 Data Drop-Down Menu

An additional drop-down menu (Math Operation drop-down menu) provides you with a selection of two different mathematical operations, which can be performed in the comparison: **difference** and **ratio**.

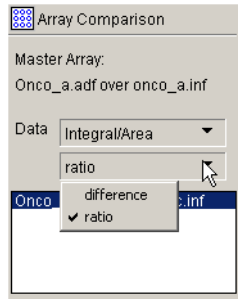


Figure 2-68 Math Operation Drop-Down Menu

Normalization Control Panel

For the comparability of the results of two arrays, a normalization of an individual result set is necessary. The **Normalization** control panel provides you with tools for defining reference dots. The values of these dots are then used for normalization calculations.

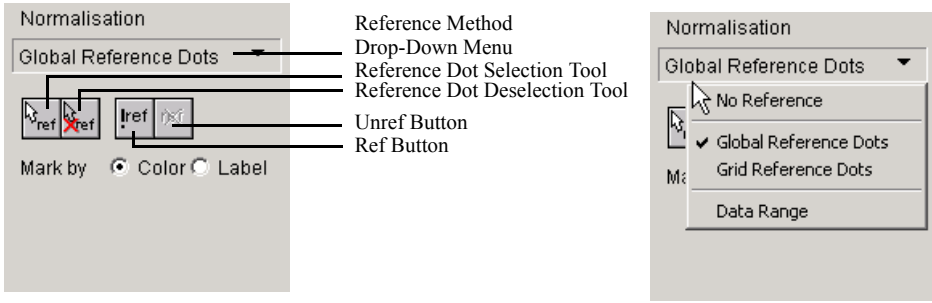


Figure 2-69 Reference Definition Section Overview

For details, see the Normalization section of this manual.

Ratio Comparison Control Panel

The **Ratio Comparison** control panel provides options for calculating the ratios of selected data.

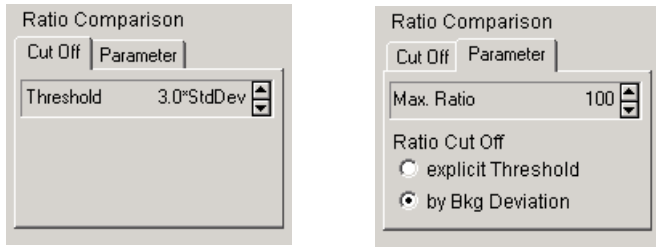


Figure 2-70 Ratio Comparison Control Panel (left: Cut Off Tab, right: Parameter Tab)

Calculating ratios of data values within background range results in biased expression values, since all measured data values exhibit a certain amount of random error due to the inherent bias of the cloning, filter production, filter processing, spotting and scanning procedures. Therefore, values within background range oscillate around 0. Forming ratios using those biased background range values just yields results comprised entirely of biases. To filter such biased ratios, you can specify thresholds for the background range via the **Cut Off** and **Parameter** tabs. All ratio values calculated from master and client values below these background thresholds will be processed using a special procedure described in the Reviewing Array Comparison Data chapter of this manual.

The default settings of the **Cut Off** tab provides you with a single **Threshold** spin box for specifying both the background threshold for the master and the background threshold for the client using a multiple of the standard deviation of the spots' background values. The default cut off threshold value is set to 3 standard deviations, which has proved to be quite usable in the majority of cases.

The **Parameter** tab allows you to change the default settings for the threshold determination. Just select the **Explicit Threshold** radio button and enter separate cut off threshold values for the master and the client on the **Cut Off** tab (if you select **explicit Threshold**, the appearance of the **Cut Off** tab changes, revealing spin boxes for specifying separate cut off values for the master and the client).

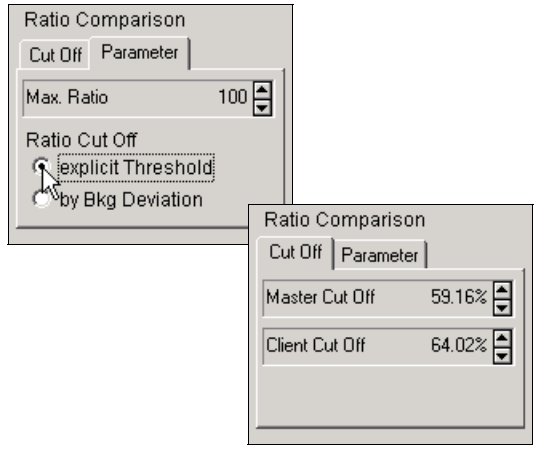


Figure 2-71 Setting Explicit Threshold

Setting a **Max Ratio** value (the highest ratio value possible) can be very useful, if you want to export the comparison data to another program post-processing these data (e.g. for cluster analysis). Since these programs usually accept numerical data only, and since calculation of ratios of values within background range does not yield reasonable results, Array Compare sets the ratio value to **Max Ratio**, if the ratio calculation results in infinity. In the majority of cases, it is reasonable, to set the **Max Ratio** value to 100.

Display Control Toolbox

This toolbox is used to control the appearance of the images on the screen.

To display the **Display Control** toolbox:

On the main menu, click **Evaluation**, and then click **Display Control**. Alternatively you can select the **Display Control** button on the toolbar.

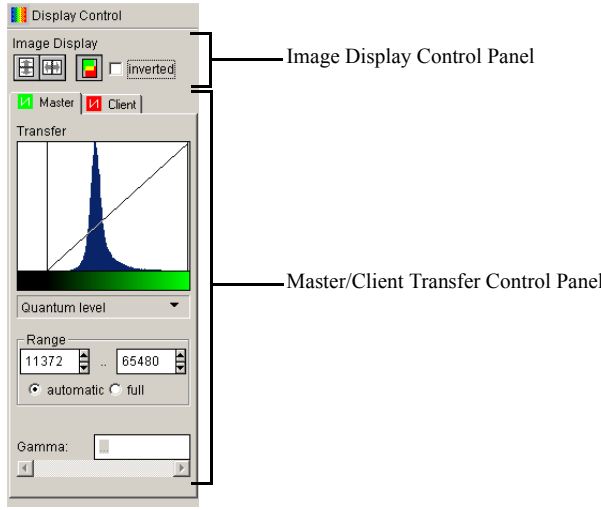


Figure 2-72 Display Control Toolbox Overview

Image Display Control Panel

With the **Image Display** control panel of the toolbox you can specify the arrangement of the two **Images** window panes by clicking the appropriate button.

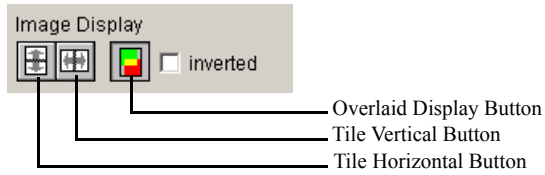


Figure 2-73 Image Display Control Panel Overview

The **Tile Horizontal**, **Tile Vertical** and **Overlaid Display** buttons correspond to the **Tile Horizontal**, **Tile Vertical** and **Overlaid Display** options of the **Images** window's shortcut menu (see Shortcut Menu of the Images Window section above). As mentioned in the Shortcut Menu of the Images Window section, the non-overlaid display types use grayscale for displaying the images, whereas the **Overlaid Display** option shows the two

images superimposed on each other in false color display. In addition, if the **Overlaid Display** option is active, you can choose to display the images in inverted false colors, which is recommended for print outs. Just check the **Inverted** option.

Master/Client Transfer Control Panel

The transfer for the master and the client images are adjustable separately. To switch between the transfer setting for master and client array, click the appropriate tab on the Master/Client Transfer control panel.

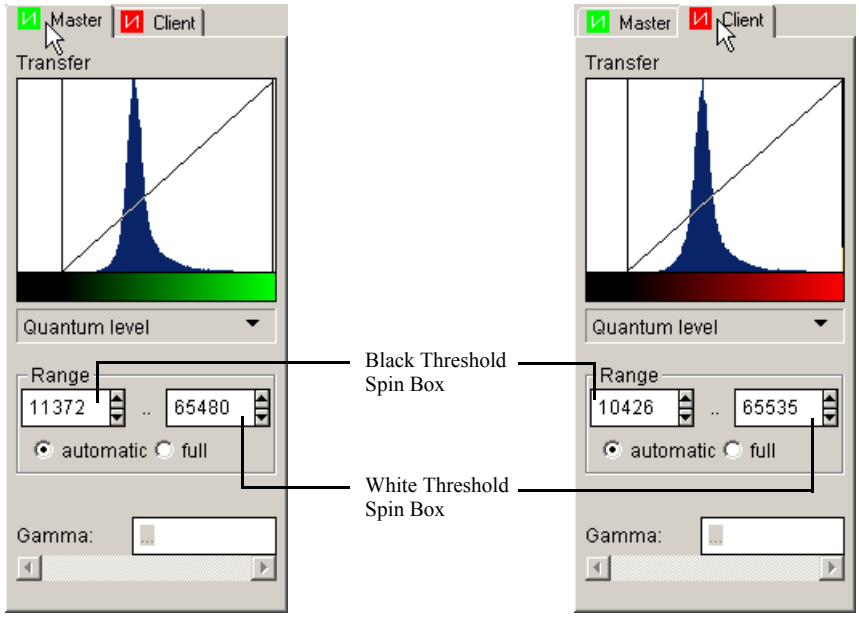


Figure 2-74 Master/Client Transfer Control Panel (Left: Master Tab, Right: Client Tab)

The histogram provided on the Master/Client Transfer control panel is a representation of the number of pixels y-axis) that have the same intensity (x-axis). The vertical line on the left-hand side of the histogram represents the black threshold, the vertical line on the right-hand side the white threshold. The actual values (in this example: 11372 and 65480) are also shown in the Black Threshold and White Threshold spin boxes on the **Range** panel of the Master/Client Transfer control panel. These values can be changed by moving the threshold indicators (vertical lines) in the histogram or by entering the values in the spin boxes. The actual transfer curve is displayed between the threshold indicators in the histogram. The transfer curve determines which original intensity (x-axis) will be

represented by which intensity on the screen (y-axis). The drop-down menu directly below the histogram gives access to different transfer curves (**Quantum Level**, **Gamma**, **S-Curve**, **PSL**).

The histogram's shortcut menu provides different views of the histogram window (**Full**, **Reduced**, **Enlarge**, **Enlarge All**). The **automatic** or the **full** radio buttons can be selected to get *automatic contrast enhancement* or *full scale representation*, respectively.

The **Gamma** value is used to change the incline of the Gamma and S-Curve. A Gamma value of 1 gives rise to a linear transfer in these curve modes. A value higher than 1 will enhance the representation of faint signals, whereas a value below 1 will enhance the high intensity values.

Array Schematic Toolbox

With the **Array Schematic** toolbox you can configure the settings for the schematic analysis of the array comparison.

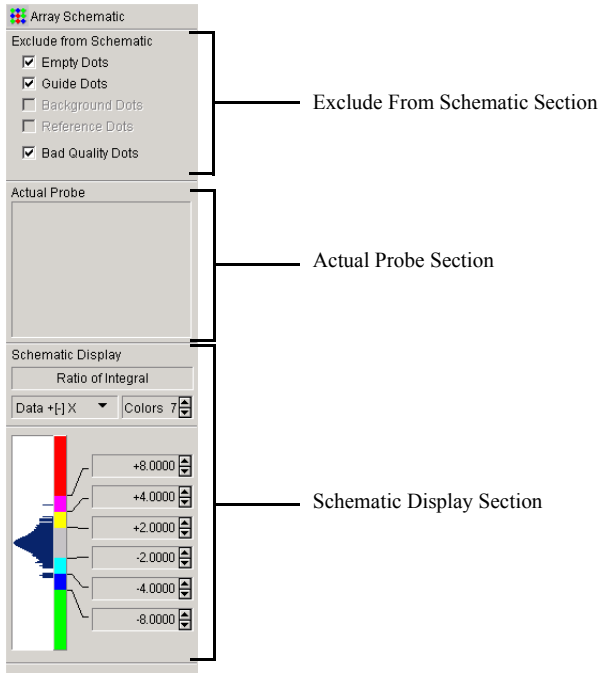


Figure 2-75 Array Schematic Toolbox Overview

Settings made via the **Array Schematic** toolbox affect not only the **Schematic Display** window, but also the **Schematic Histogram** as well as the **Array Correlation** plot. Furthermore, the ratio and expression values recorded in the **Array Result Table** are affected by the **Array Schematic** toolbox settings too.

Exclude From Schematic Section

In the **Exclude from Schematic** section of the **Array Schematic** toolbar you can select some dot types for exclusion from the schematic analysis. These types are: **empty dots**, **guide dots**, and **bad quality dots**.

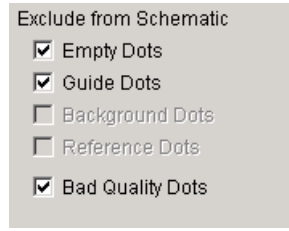


Figure 2-76 Exclude From Schematic Section

Values excluded via the **Exclude from schematic** option appear in gray in the **Schematic Display** window and are marked with a cross to avoid confusion with “real” gray scale values.

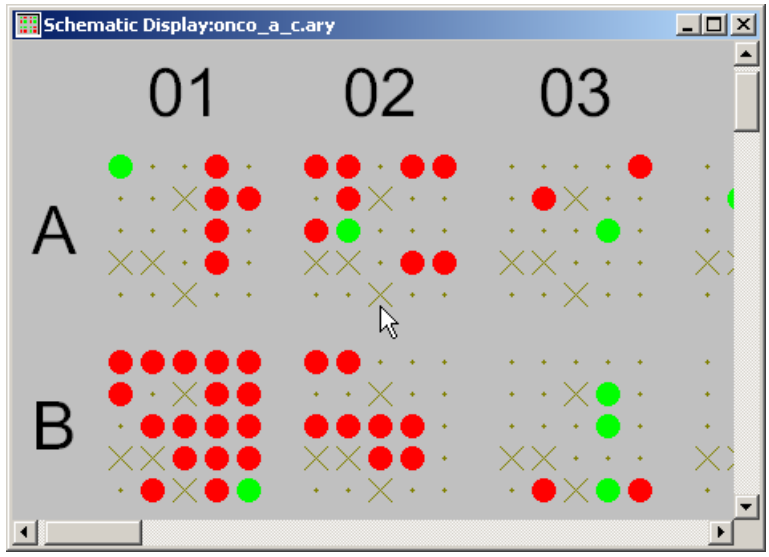


Figure 2-77 Dots Excluded From Schematic

Consequently, they are also excluded from the **Schematic Histogram** calculation and are not displayed in the **Array Correlation** plot.

Actual Probe Section

In the message box below the **Exclude from schematic** section, the name of the selected dot as stated in the **Array Result Table** is shown together with its label and the value for the measured data (selected in the **Data** drop-down menu of the **Array Comparison** toolbox).

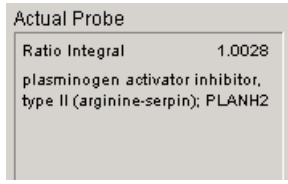


Figure 2-78 Actual Dot Section

Schematic Display Section

The **Schematic Display** section is used to configure the display options for the **Schematic Display** window.

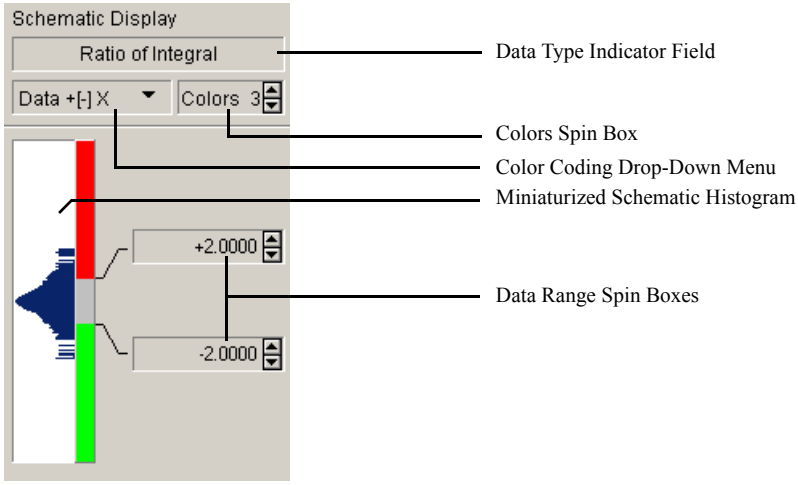


Figure 2-79 Schematic Display Section Overview

In the Data Type Indicator Field, the data type to be displayed in the **Schematic Display** window is shown.

To configure the color output of the **Schematic Display** window, choose the number of colors (3, 5, or 7) you want the data to be reduced to using the **Colors** spin box.

Specify the color coding scheme for the data via the drop-down menu displayed to the left of the **Colors** spin box. Either the data of interest within a specified range are displayed in one of the colors (**DATA +[-] X** option) or the deviation to the mean value is coded by the colors (**MEAN +[-] SD** option).

If the **DATA +[-] X** option is selected, you can set the data range for each color by dragging the border of the respective color bar in the miniaturized **Schematic Histogram** with the mouse or by entering the explicit values into the data range spin boxes.

If the **MEAN +[-] SD** option is selected, this feature is disabled. Instead, the deviation of the spots to the mean value of the Log ratio data is given in units of 10th standard deviation (SD) of Log ratio. Enter the step size in the **SD Pitch** spin box.

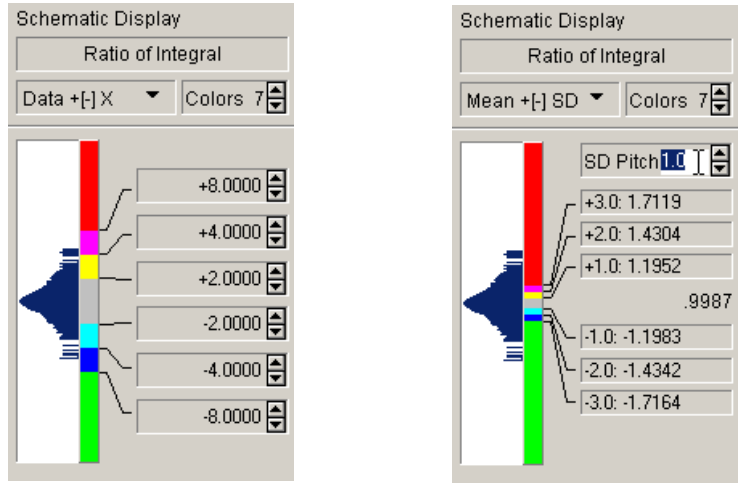


Figure 2-80 Setting Data Range (left) and Specifying Step Size (right)

Note that all data range values have to be entered (and are given) in terms of expression values (ratio values > 1 are converted to expression values +*ratio value*, whereas ratio values < 1 are converted to expression values -1/*ratio value*) into the spin boxes. For the ratio comparison of means, the mean values of the Log ratio data, given in units of 10th standard deviation (SD) of Log ratio, are shown adjacent to the respective expression values.

Array Analysis Table Toolbox

With the **Array Analysis Table** toolbox you can configure the settings for the **Analysis Table** window.

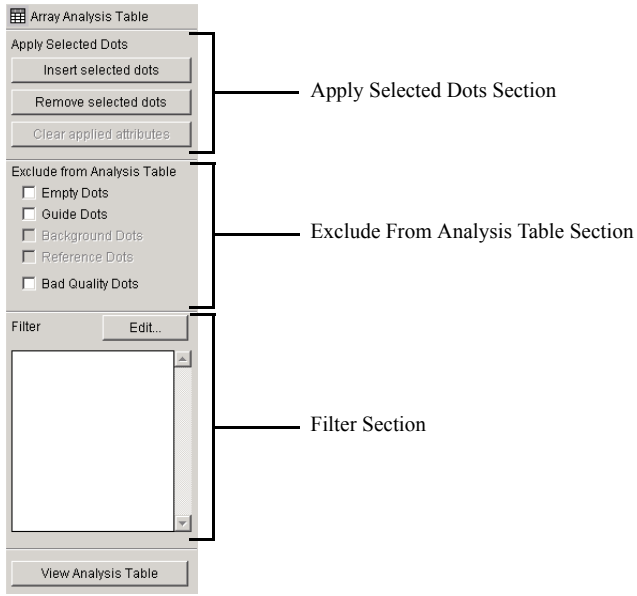


Figure 2-81 Array Analysis Table Toolbox Overview

Apply Selected Dots Section

The controls of this panel are used to manually add values to or remove values from the **Analysis Table**.

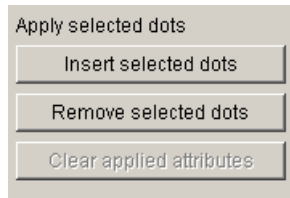


Figure 2-82 Apply Selected Dots Section

Exclude From Analysis Table Section

In the **Exclude from analysis table** section of the **Array Analysis Table** toolbox you can select some dot types for exclusion from the **Analysis Table**. These types are: **empty dots**, **guide dots**, and **bad quality dots**.

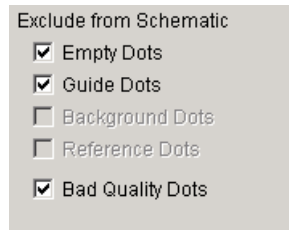


Figure 2-83 Exclude From Analysis Table Section

Filter Section

This panel allows you to guide the automatic filtering process for analysis result values provided by AIDA Array Compare.

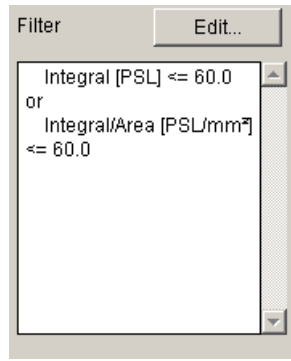


Figure 2-84 Filter Section With Rules Assigned

The automatic result value filtering process is based on rules defined by the user. There are no built-in default filtering rules, so unless you define a specific rule for filtering, no automatic filtering is applied. The filter to be specified consist of a list of conditions (**Alternatives**) concatenated by an OR operator. An Alternative is a AND-linked list of conditionals for evaluating the data values of a particular spot.

The filter rules you define are *Permission* rules: Values that *do not* meet the conditions stated in the rules are blocked from being displayed in the **Analysis Table** window.

To enter a rule statement:

1 Click the **Edit** button on the **Filter** panel.

The **Array Analysis Table Clauses** dialog box appears.

2 On the **Alternatives** panel, click **Add**.

This adds the phrase **1. Clause is:** to the **Alternatives** list panel.

3 Then click the **Add** button on the Clauses panel.

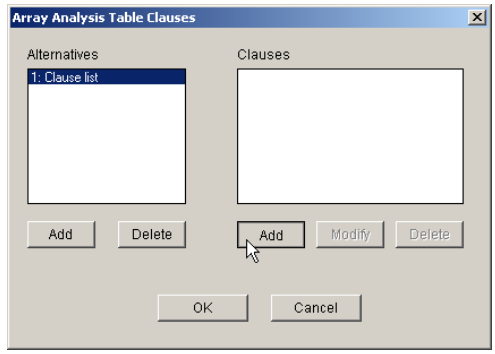


Figure 2-85 Adding rule clauses

This opens the **Analysis Table Clauses** dialog box, which allows you to define your rule expression.

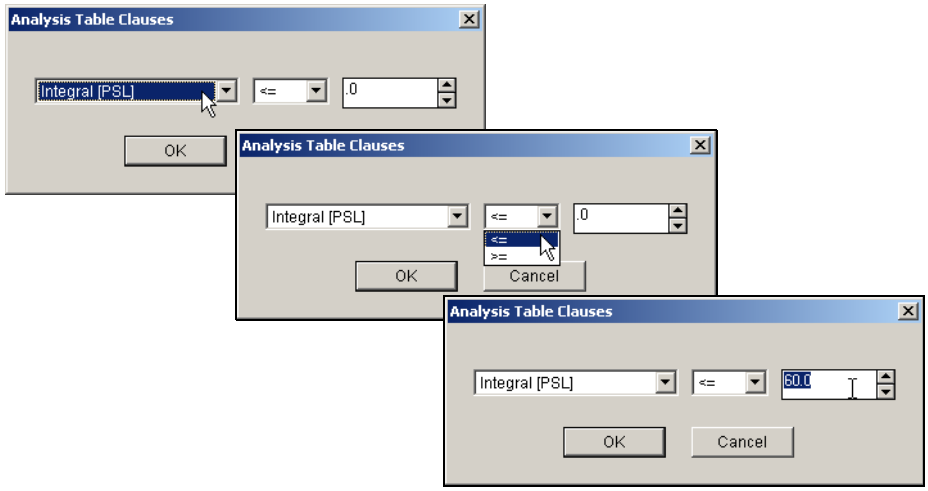


Figure 2-86 Specifying Rule Expression

Publishing Layout Toolbox

The **Publishing Layout** toolbox is used to configure the various print settings provided by Array Compare. In contrast to the settings performed via the **Print** command of the **File** menu, the **Protocol Print** options are permanently stored with the evaluation data.:

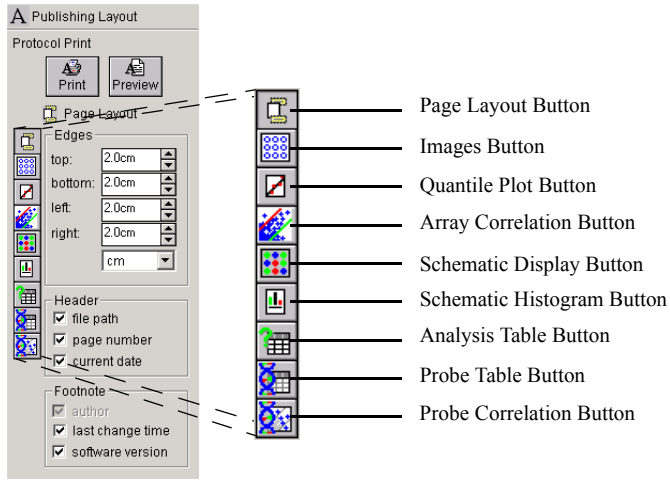


Figure 2-87 Protocol Print Toolbar

Print Options for Page Layout

Selecting the Page Layout button, you can specify the layout for the printout.

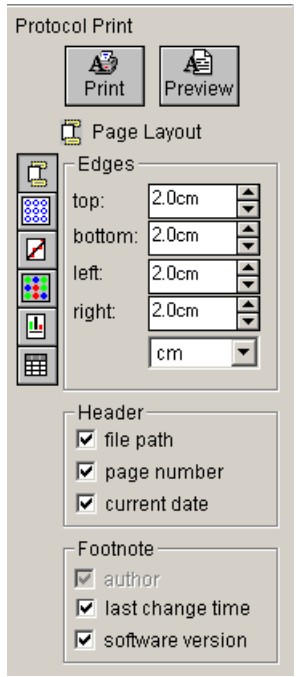


Figure 2-88 Protocol Print Section (Page Layout)

In the **Edges** part, you can specify the margins (in cm or inch) for the printout.

In the **Header** part, you can select whether to include the file path, the page number and/or the current date in the header of the printouts.

In the **Footnote** part, **author**, **last change time** and **software version** can be selected to appear in a footnote.

Print Options for Images Window

Selecting the **Images** option, you can specify the paper orientation for the printout as well as the items you want to print.

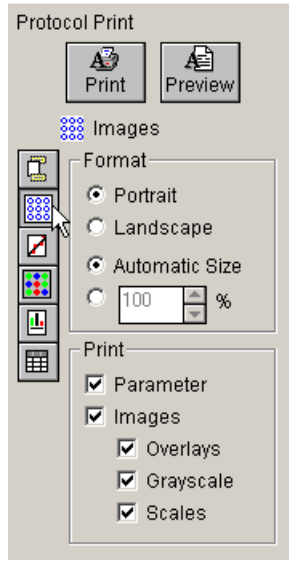


Figure 2-89 Protocol Print Section (Images)

The printout result depends on the current **Image Display** settings used in the **Display Control** toolbox, i.e., for example, if you have chosen **Overlaid Display** in the **Display Control** toolbox, the images are printed overlaid accordingly. If you have set **Image Display** to **Tile Vertical** or **Tile Horizontal**, the images are printed one after the other. In the **Format** part, select the paper orientation for the printout (**Portrait** or **Landscape**). The size of the image can be automatically adjusted to fit the page (**Automatic Size**). Alternatively you can enter the zoom factor in percent in the spin box located directly below the **Automatic Size** radio button.

In the **Print** section, the **Parameter** checkbox enables the printing of the data and comment which are found in the **File Information** dialog box. Check **Overlays**, if you want to print the images with the array overlays superimposed on them. Selecting **Grayscale** prints a bar next to the image, showing the representation of various signal intensities as gray scales in the way they were set for the image. **Scales** prints a bar next to the image that shows the length representation in the image.

Print Options for Quantile Plot

The **Quantile Plot** button on the **Protocol Print** toolbar allows you to configure the layout setting for the **Quantile Plot** window. You can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** section.The **Graphic** option in the **Print** section enables or suppresses the print output of the graphic displayed in the **Quantile Plot** window.

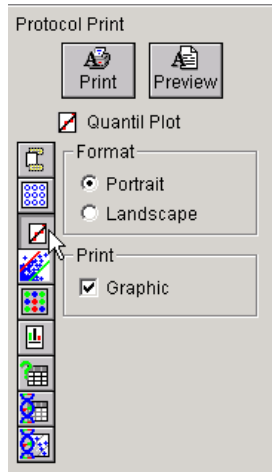


Figure 2-90 Quantile Plot Print Options

Print Options for Array Correlation

The **Array Correlation** button on the **Protocol Print** toolbar allows you to configure the layout setting for the **Array Correlation** window. You can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** section.The **Graphic** option in the **Print** section enables or suppresses the print output of the graphic displayed in the **Array Correlation** window.

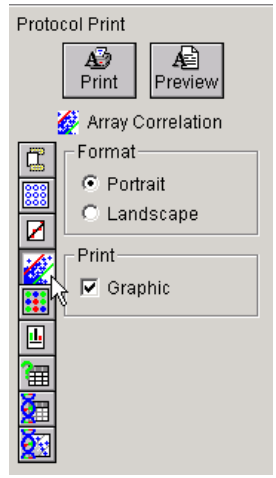


Figure 2-91 Array Correlation Print Options

Print Options for Schematic Display

To configure the layout setting for the **Schematic Display** window, choose the **Schematic Display** button provided by the **Protocol Print** toolbar. As for **Array Correlation**, you can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** section.The only option in the **Print** section, **Graphic**, enables or suppresses the print output of the **Schematic Display** window.

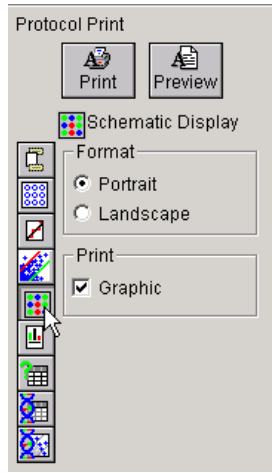


Figure 2-92 Schematic Display Print Options

Print Options for Schematic Histogram

The **Schematic Histogram** button on the **Protocol Print** toolbar allows you to configure the layout setting for the **Schematic Histogram** window. You can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** section.The **Graphic** option in the **Print** section enables or disables the print output of the graphic displayed in the **Schematic Histogram** window.

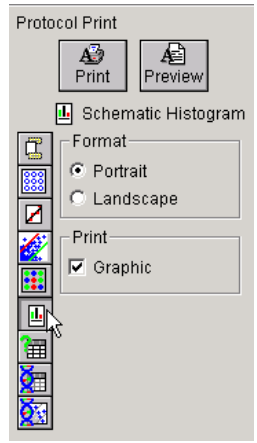


Figure 2-93 Schematic Histogram Print Options

Print Options for Array Analysis Table

Clicking the **Array Analysis** button switches to the layout setting controls for the **Array Analysis Table** window. To select the paper orientation (**Portrait** or **Landscape**), click the appropriate radio button in the **Format** section. In the **Print** section the scope for the print output can be specified. To enable printing of the table, check the **Table** box and specify the appropriate output extent using the **Full Table**/**Rows above** radio buttons.

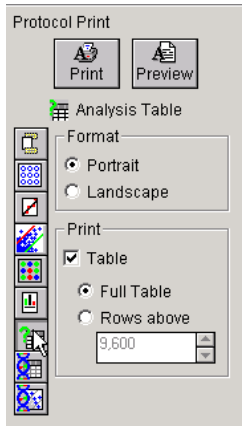


Figure 2-94 Array Analysis Table Print Options

Print Options for Probe Table

Clicking the **Probe Table** button switches to the layout setting controls for the **Probe Table** window. To select the paper orientation (**Portrait** or **Landscape**), click the appropriate radio button in the **Format** section. In the **Print** section the scope for the print output can be specified. To enable printing of the table, check the **Table** box and specify the appropriate output extent using the **Full Table**/**Rows above** radio buttons.

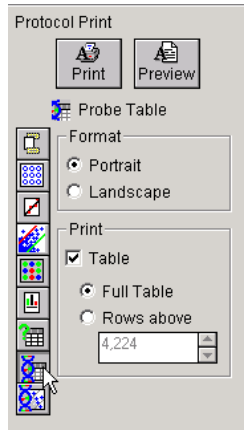


Figure 2-95 Probe Table Print Options

Print Options for Probe Correlation

The **Probe Correlation** button on the **Protocol Print** toolbar allows you to configure the layout setting for the **Probe Correlation** window. You can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** section.The **Graphic** option in the **Print** section enables or suppresses the print output of the graphic displayed in the **Probe Correlation** window.

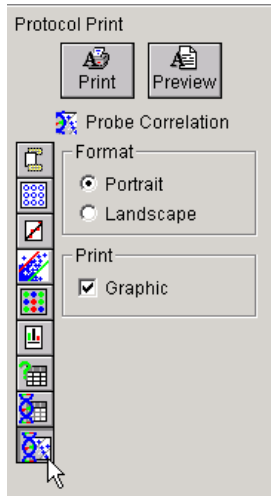


Figure 2-96 Probe Correlation Print Options

Print

In AIDA Array Compare there are two ways for printing your image and evaluation data:

1. Select the **Print** option from the **File** menu, or
2. use the **Protocol Print** section on the **Publishing Layout** toolbox.

The **Print** command of the **File** menu allows you to print your data 'on the fly' without permanently storing the layout/printing settings, whereas the **Protocol Print** panel provides you with a complete set of printing features which includes saving the setting with the data file for reasons of reproducibility.

Selecting the **Print** option from the **File** menu opens the **Print Array Compare** dialog box, allowing you to configure the printout to contain only the overlays, results and graphs that are currently needed.

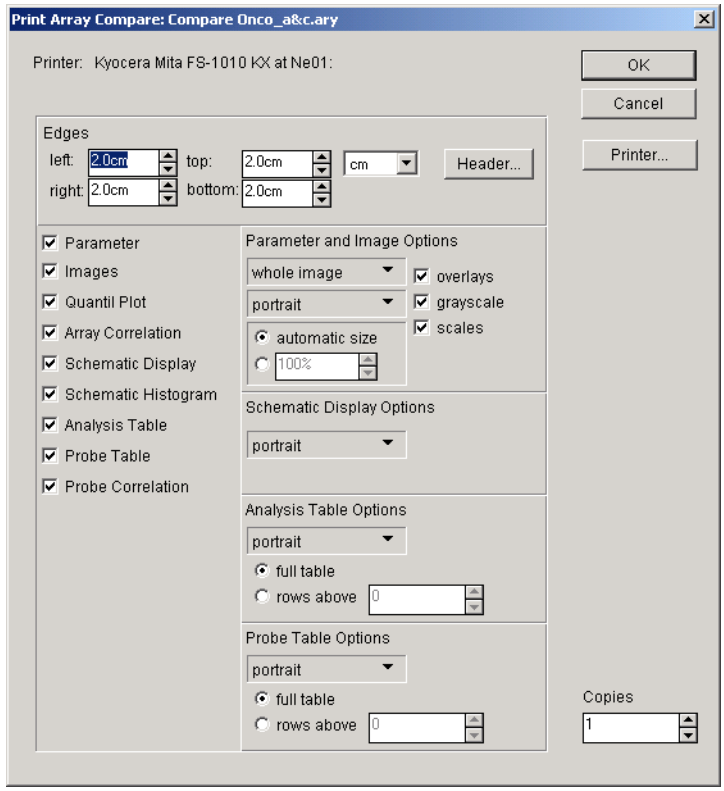


Figure 2-97 Print Array Metrix Dialog Box

Click the check boxes for the various items to enable or disable them. Select the number of copies, you want to print.

The margins of the printout can be selected in the **Edges** part of the dialog box. The **Header** button opens the following dialog box:

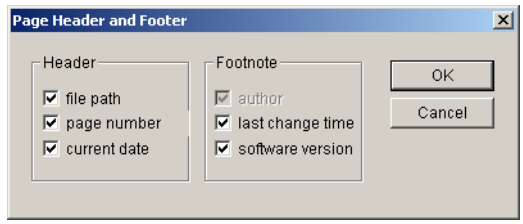


Figure 2-98 Page Header and Footer Dialog Box

Here the **Header** and **Footer** of the report can be configured to contain various image and evaluation specific data.

The **Parameter** (data overlays), the **Images**, **Array Correlation**, **Schematic Display**, **Array Histogram** and **Array Analysis Table** information can be selected in the left part of the **Print Array Compare** dialog box.

In the right part of the dialog box (**Parameter and Image Options** section) you can select **Whole Image**, or **Window Content** from the first drop-down menu. Choose **Landscape** or **Portrait** format from the second drop-down menu. Further, the printing of **Overlays**, **Grayscales**, **Scales**, and **Overexposed** can be enabled by checking the respective options. The printout can be configured to automatically fit to the page, or the relative size of the image representation can be selected (in %). In the **Analysis Table Options** and **Probe Table Options** sections you can specify to print the entire tables (**full table**) or just a part of them (**rows above**) as well as the paper orientation (**landscape** or **portrait**). The paper orientation can also be specified for the **Schematic Display** output using the drop-down menu in the **Schematic Display Options** section.

Preview

As for printing, in AIDA there are two ways of previewing pages before they are actually printed:

1. Using the **Print Preview** option of the **File** menu, or
2. using the **Preview** button on the **Publishing Layout** toolbox.

The **Print Preview** option of the **File** menu opens the **Preview** dialog box, which allows you to specify the layout settings for the preview. The options presented here are the same as in the **Print Array Compare** dialog box and those selectable from the various sections of the **Publishing Layout** toolbox. However, they are not stored with the data file.

The **Preview** option of the **Publishing Layout** toolbox allows you to preview all pages as configured in the **Publishing Layout** toolbox before they are actually printed. Clicking the **Preview** button opens the **Preview** window directly, since all layout setting are already made through the **Protocol Print** panel.

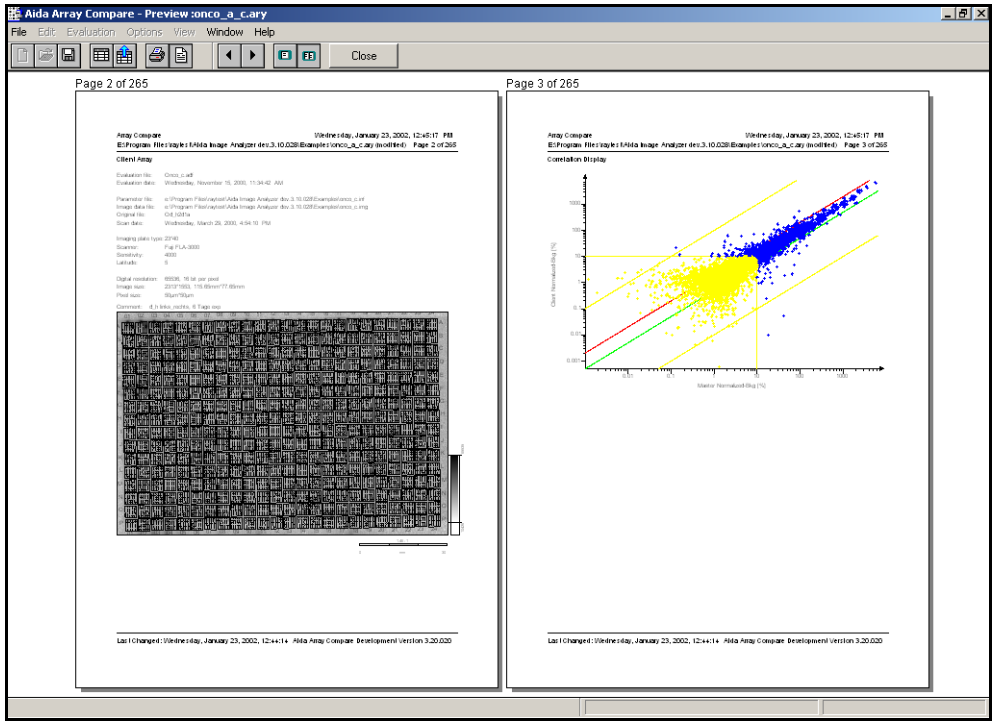


Figure 2-99 Preview Example

The active zoom function allows to closely look at some details and works as described for the Zoom tool (left mouse button: zoom in, right mouse button: zoom out).

Besides the standard features, the toolbar of the **Preview** window provides the following functions:

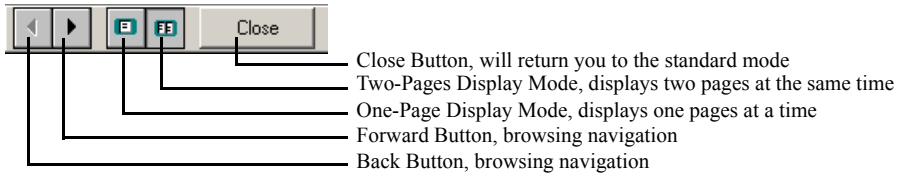

Close
Close Button, will return you to the standard mode
Two-Pages Display Mode, displays two pages at the same time
One-Page Display Mode, displays one pages at a time
Forward Button, browsing navigation
Back Button, browsing navigation

Figure 2-100 Toolbar of the Preview Window

Export and Import

In AIDA Array Compare, array comparisons as well as the content of all windows can be exported to a specific file.

Exporting Images

To export the content of the **Images** window in a raster graphic format, choose the **Images** option from the **Export** cascading menu of the **File** menu or select **Copy Content** from the **Edit** menu. The **Images Export** dialog box opens asking you to specify a name and data format (Windows Bitmap or CompuServe GIF format) for the image export file.

After you confirmed the export by clicking the **Save** button, the **Image Export Options** dialog box appears, allowing you to set further option for the image file export.

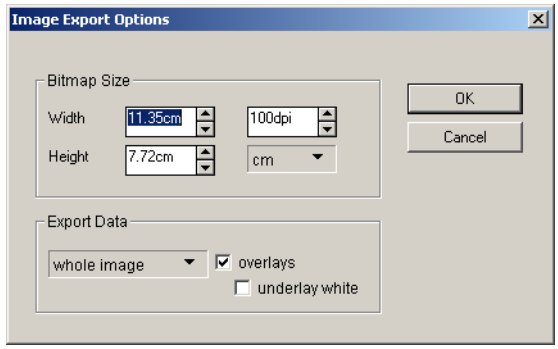


Figure 2-101 Image Export Options Dialog Box

Depending on the purpose of the export bitmap, you can select the size and resolution of the resulting bitmap.

The raster graphic is exported in the Windows Bitmap or the CompuServe GIF format.

Note that the two images are exported as one 'overlaid' image. Use AIDA Image Analyzer to export each of the two images separately.

Exporting Quantile Plots

To export the **Quantile Plot** display:

On the **File** menu, click the **Export** menu item and then select **Quantile Plot** from the cascading menu. In the **Quantile Plot Export** dialog box that appears enter a name for the export file.

Complete the export action by clicking the **Save** button. The data are saved to a file in the Windows Metafile format.

Exporting Array Results

To export the **Array Result Table**:

On the **File** menu, click the **Export** menu item and then select **Array Results** from the cascading menu. In the **Array Results Export** dialog box that appears enter a name for the export file.

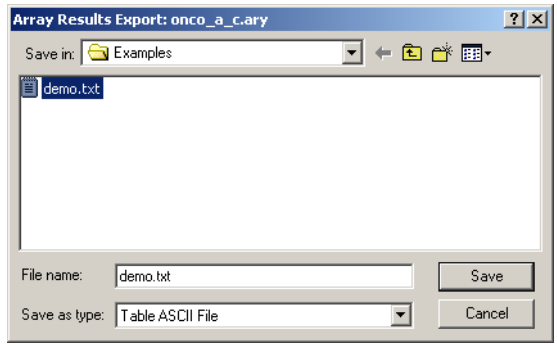


Figure 2-102 Array Results Export Dialog Box

After you confirmed the export by clicking the **Save** button, the **Array Results Export Options** dialog box appears, allowing you to set further option for the export of the array results.

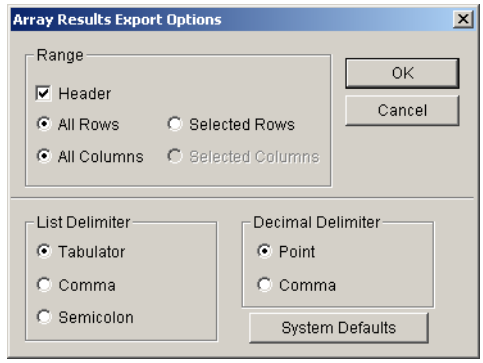


Figure 2-103 Array Results Export Options Dialog Box

You can specify the **Range** of the exported rows and columns as well as the **List Delimiter** and **Decimal Delimiter** in the respective panels of the dialog box.

Complete the action by clicking the **OK** button. The data are saved to a simple ASCII (text) file.

Exporting Array Correlation

To export the **Array Correlation** display:

On the **File** menu, click the **Export** menu item and then select **Array Correlation** from the cascading menu. In the **Correlation Display Export** dialog box that appears enter a name for the export file.

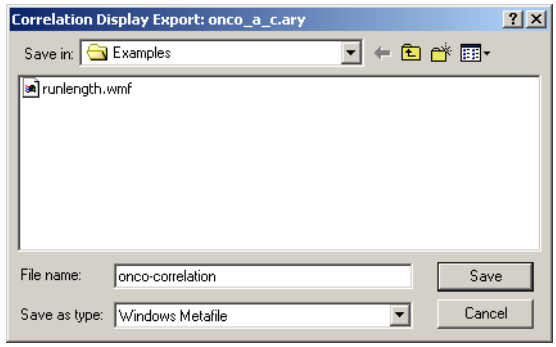


Figure 2-104 Correlation Display Export Dialog Box

Complete the export action by clicking the **Save** button. The data are saved to a file in the Windows Metafile format.

Exporting Schematic Display

To export the **Schematic Display**:

On the **File** menu, click the **Export** menu item and then select **Schematic Display** from the cascading menu. In the **Schematic Display Export** dialog box that appears enter a name for the export file.

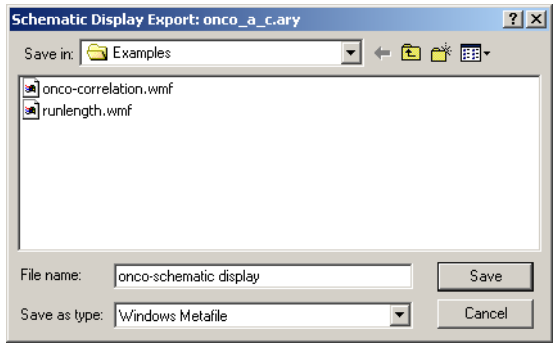


Figure 2-105 Schematic Display Export dialog box

Complete the export action by clicking the **Save** button. The data are saved to a file in the Windows Metafile format.

Exporting Schematic Histogram

To export the **Schematic Histogram**:

On the **File** menu, click the **Export** menu item and then select **Schematic Histogram** from the cascading menu. In the **Schematic Histogram Export** dialog box that appears enter a name for the export file.

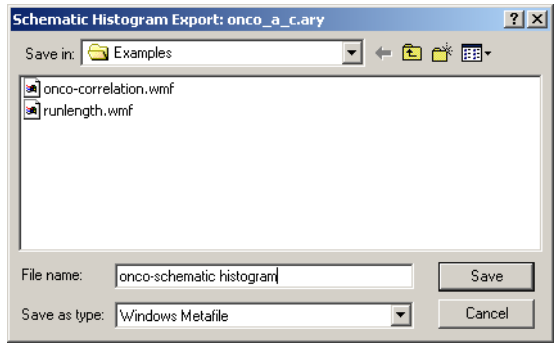


Figure 2-106 Schematic Histogram Export dialog box

Complete the export action by clicking the **Save** button. The data are saved to a file in the Windows Metafile format.

Exporting Analysis Table

To export the **Analysis Table**:

On the **File** menu, click the **Export** menu item and then select **Analysis Table** from the cascading menu. In the **Analysis Table Export** dialog box that appears enter a name for the export file.

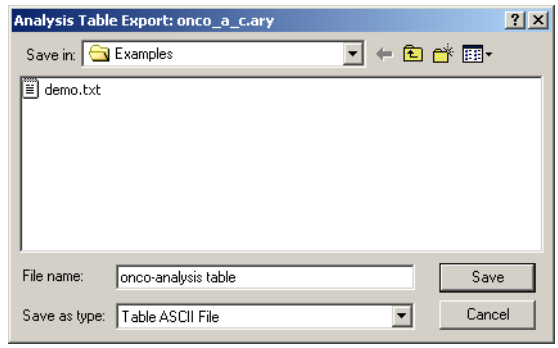


Figure 2-107 Analysis Table Export Dialog Box

After you confirmed the export by clicking the **Save** button, the **Analysis Table Export Options** dialog box appears, allowing you to set further option for the export of the analysis table.

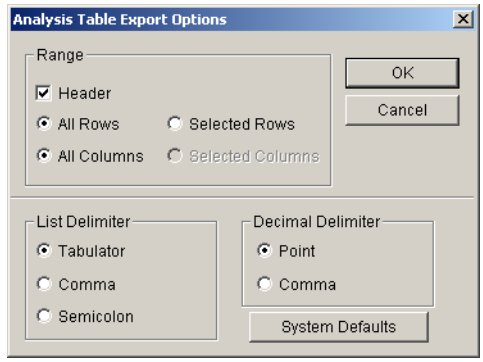


Figure 2-108 Analysis Table Export Options Dialog Box

You can specify the **Range** of the exported rows and columns as well as the **List Delimiter** and **Decimal Delimiter** in the respective panels of the dialog box.

Complete the action by clicking the **OK** button. The data are saved to a simple ASCII (text) file.

Exporting Probe Table

To export the **Probe Table**:

On the **File** menu, click the **Export** menu item and then select **Probe Table** from the cascading menu. In the **Probe Table Export** dialog box that appears enter a name for the export file.

After you confirmed the export by clicking the **Save** button, the **Probe Table Export Options** dialog box appears, allowing you to set further option for the export of the probe table.

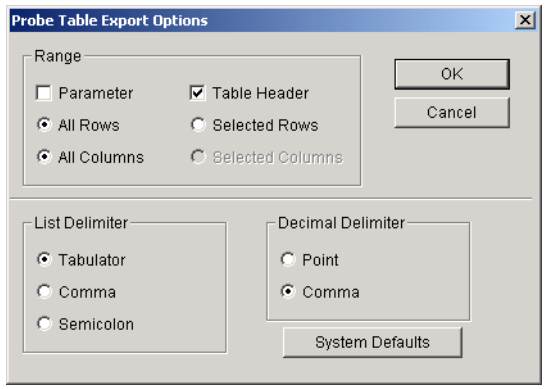


Figure 2-109 Analysis Table Export Options Dialog Box

You can specify the **Range** of the exported rows and columns as well as the **List Delimiter** and **Decimal Delimiter** in the respective panels of the dialog box.

Complete the action by clicking the **OK** button. The data are saved to a simple ASCII (text) file.

Exporting Probe Correlation

To export the **Probe Correlation** display:

On the **File** menu, click the **Export** menu item and then select **Probe Correlation** from the cascading menu. In the **Probe Correlation Export** dialog box that appears enter a name for the export file.

Complete the export action by clicking the **Save** button. The data are saved to a file in the Windows Metafile format.

Importing Array Evaluations

To add an array client to the master for comparison, click the **File** menu and then select **Array Evaluation** in the **Import** cascading menu. Choose the array file, you want to import from the file list in the **Array Evaluation Import** dialog box that appears and click **Open**.

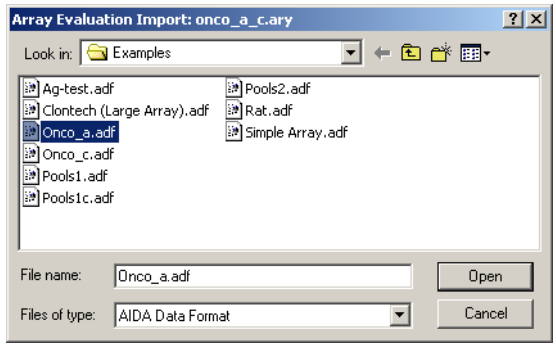


Figure 2-110 Array Evaluation Import Dialog Box

Alternatively, you can click the **Add** button on the **Array Comparison** toolbox to open the **Array Evaluation Import** dialog box and select your client array file.

Importing Name Tables

The **Name Table Import** option is intended for easy import of names and ID numbers of samples like clones, sequences, oligos etc. Usually, importing name tables is part of the array template definition procedure done in Array Metrix. Nevertheless, you can add additional information or replace existing information by importing them from external table sources using Array Compare's import name table options.

As in Array Metrix, you can import up to four columns from a source name table. In order to properly insert the rows of the source columns into matching row slots of the target table (Array Result Table), the position of the particular label column in the source table (import table) must be specified.

Array Metrix provides four methods for associating table rows:

1. Import by Global Label
2. Import by Hierarchic Label
3. Import by Global Position
4. Import by Hierarchic Positions

The **Import by Global Label** methods associates rows using the **Label** column, **Import by Hierarchic Label** uses the **Label 1**/**Label 2**/**Label 3** columns, I**mport by Global Position** utilizes the **Row Index** and **Column Index** columns, whereas the **Import by Hierarchic Positions** method associates source and target rows via the **Row 1**/**Row 2**/**Row 3** and **Column 1**/**Column 2**/**Column 3** columns.

To import Name Tables to the Array Result Table:

1. Click the **Import** menu item on the **File** menu and then choose **Name Table** from the cascading menu.

   The **Name Table Import** dialog box appears.

2. Select the file you want to import from the list of available files and click **Open**.

   The file has to be in the Array Name Table or Table ASCII File format.

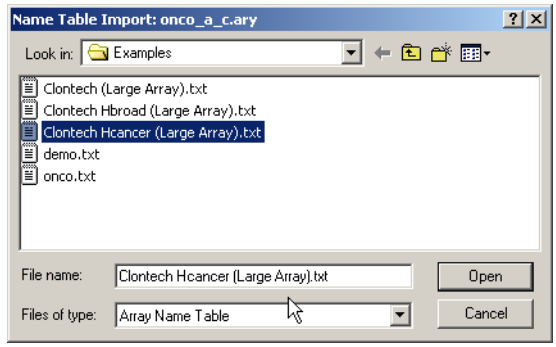


Figure 2-111 Name Table Import Dialog Box

The **Import Name Table** dialog box appears allowing you to specify the options for the name table import via spin boxes and tabs. In the lower part of the dialog box, the import source table is displayed.

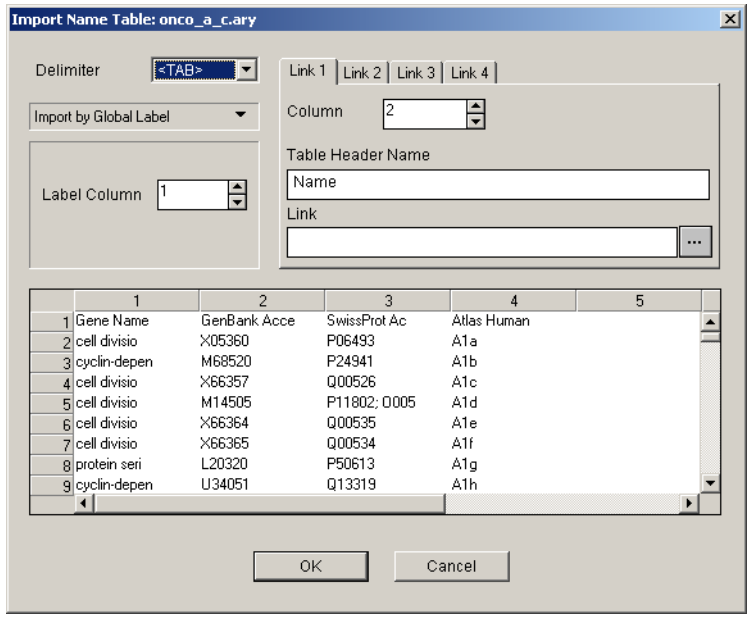


Figure 2-112 Import Name Table Dialog Box

3 Select the type of delimiter (TAB, comma, semicolon or SPACE) from the **Delimiter** drop-down list.

4 Specify the import method using the pull-down menu below the **Delimiter** drop-down list.

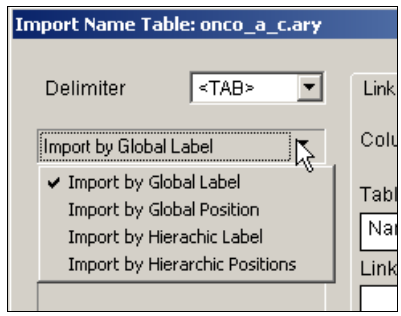


Figure 2-113 Selecting Import Method

5 Specify the associations between source table (name table to be imported) and target table (current Array Result table), depending on the import method you selected:

If you specified the **Import by Global Label** method or the **Import by Hierarchic Label** method:

- Specify the position of the label column in the source table using the **Label Column** spin box (for **Import by Global Label**) or the **1.Level Label**/**2.Level Label**/**Spot Label** spin boxes (for **Import by Hierarchic Label**).

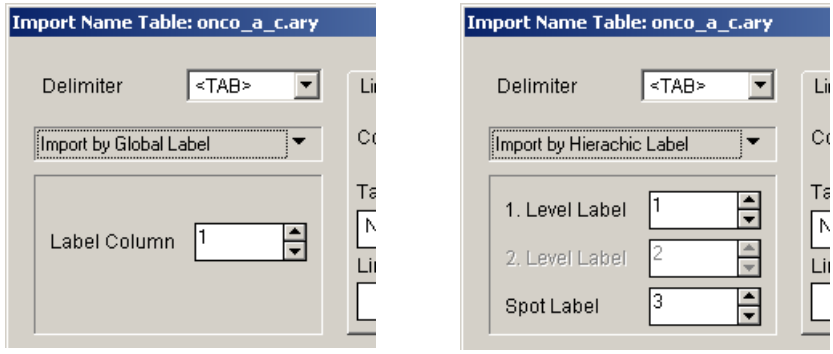


Figure 2-114 Specifying Label Column Positions

If you choose the **Import by Global Position** method or **Import by Hierarchic Positions**:

- Specify the positions of the column and row indices in the source table using the **Column Index** and **Row Index** spin boxes (for **Import by Global Position**) or the **Column** and **Row** spin boxes for **1.Level**/**2.Level**/**Spot Level** (if **Import by Hierarchic Positions** is selected).

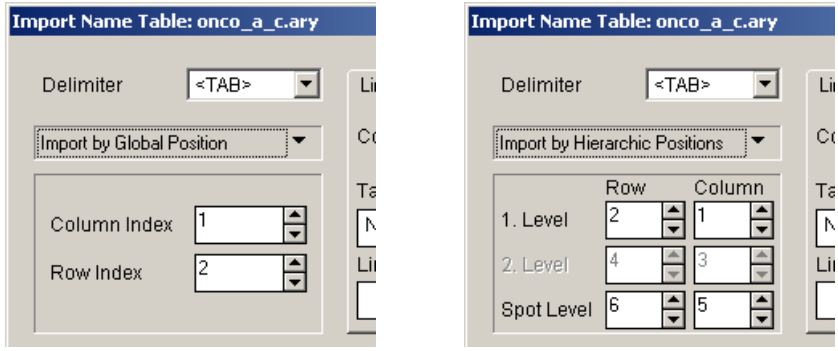


Figure 2-115 Specifying Index Positions

Using the tabbed panel of the **Import Name Table** dialog box (**Link 1**, **Link 2**, **Link 3** and **Link 4**) you can specify up to four source columns to be imported into your target table. Furthermore, the tabs allow you to enter a user-defined name for the column header of the resulting target column. In addition, the cells of imported columns (**Name** or **ID** for example) can be hyperlinked to internal or external databases, so you can search the linked databases for the name or ID by just clicking the respective cell in the **Array Result Table**. (Name and hyperlink of imported columns can adjusted via the **Settings of** command selectable from the shortcut menu of the respective column.)

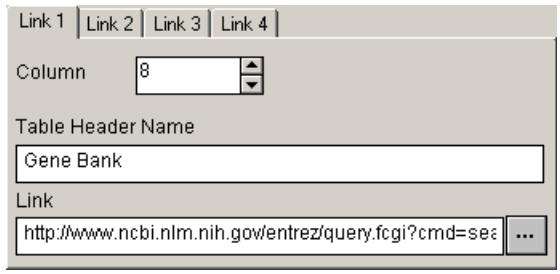


Figure 2-116 Link Panel

6 Enter the number of the source table column you want to import into your target table using the **Column** spin box of the **Link *n*** tab.

Look up the column number in the source table display shown in the lower part of the dialog box.

Note – Entering the column number 0 in the **Column** spin box of a **Link *n*** tab indicates that no source column has been selected for import on this particular tab.

---

7 Enter a name for the column to be displayed in the header of the imported column in the **Table Header Name** text box.

8 Enter the query string for database search using database-specific query syntax in the **Link** text box.

Alternatively you can select predefined/previously defined query strings from a list

a. Click the button next to the **Link** text box.

The **Table URL Links** dialog box opens.

b. Choose an entry from the list box and click **OK**.

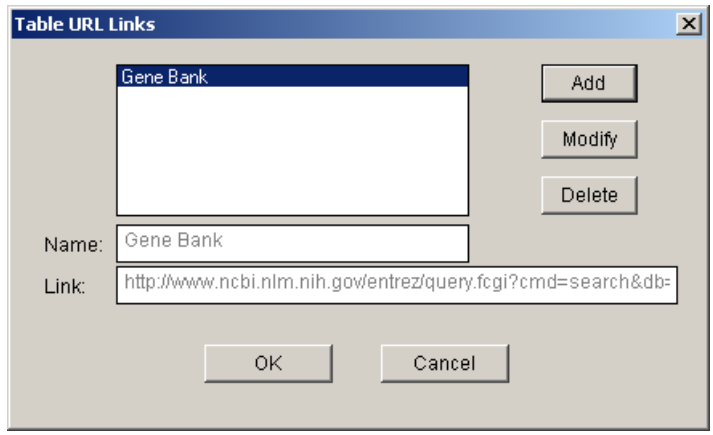


Figure 2-117 Selecting Query String From the Table URL Links Dialog Box

Comparing Arrays 3

Loading Master and Client Arrays

To load two arrays for comparison:

1 Choose **New** from the **File** menu.

The **Array Evaluation Import** dialog box appears, allowing you to select an array comparison master file.

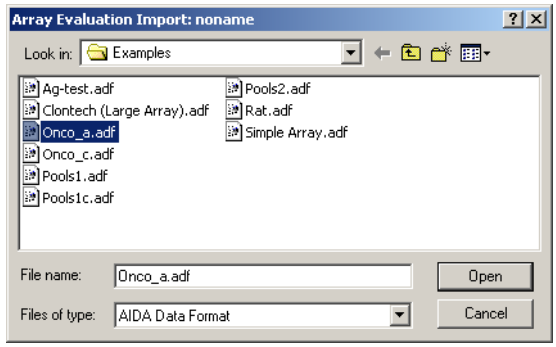


Figure 3-1 Loading Array Comparison Master File

2 Select the appropriate file and click **Open**.

The array is loaded in the first half of the bipartite **Images** window and the file name is listed below the **Master Array** heading in the **Array Comparison** toolbox.

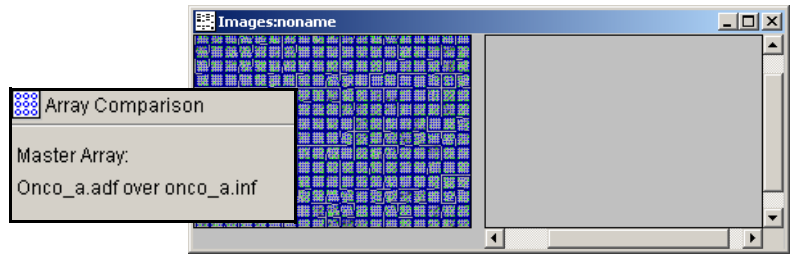


Figure 3-2 Master File Loaded

3 Perform one of the following steps to load the comparison client array:

- Select **Array Evaluation** from the **Import** cascading menu of the **File** menu.
- Click the **Add** button on the **Array Comparison** toolbox.

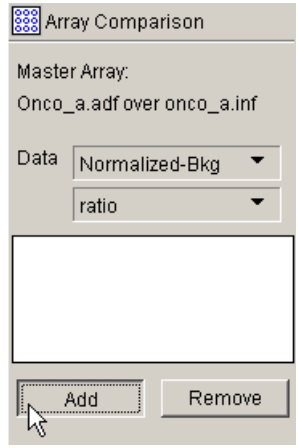


Figure 3-3 Adding Client Array

This opens the **Array Evaluation Import** dialog box.

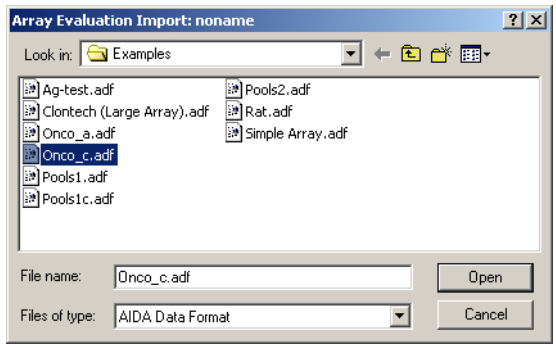


Figure 3-4 Selecting Client Array File

4 Select the appropriate client array file from the file list and click **Open**.

The comparison client is loaded into the second pane of the **Images** window.

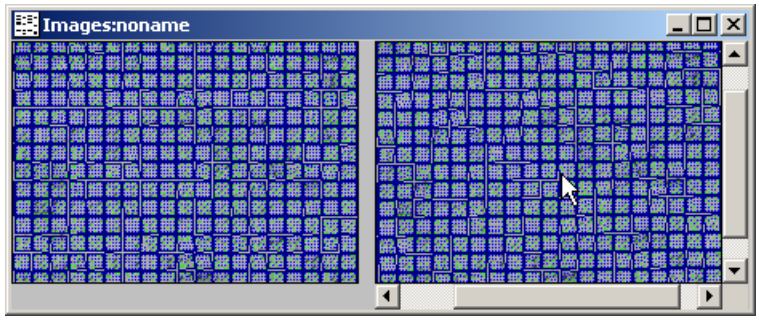


Figure 3-5 Client Loading Completed

Normalization

For the comparability of the results of the two arrays, a normalization of an individual result set is necessary. AIDA Array Compare offers three methods of normalization, which can be selected from the drop-down menu of the **Array Comparison** toolbox's **Normalization** section.

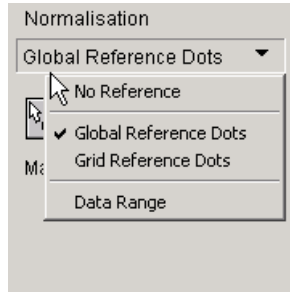


Figure 3-6 Normalization Method Drop-Down Menu

After the normalization process is completed, additional columns (**Normalized[%]**, **Normalized-Bkg[%]**, **Reference**, **Reference-Bkg**) are inserted in the **Array Result Table**. For details about this columns, see the "Array Result Table Window" section of this user's manual.

Global Reference Dots

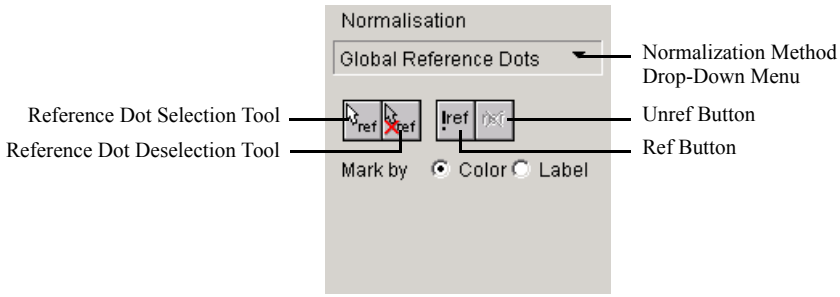


Figure 3-7 Reference Definition Section Overview

Each result (integral or integral minus background) of a spot is referred to either the mean value of all spots or to individually selected normalization spots. Normalization is done using the same reference or reference-bkg value for all spots.

To mark a reference spot (normalization spot):

1. Choose the **Selection** tool from the main toolbar.
2. Click the designated spot
3. Click the **Ref** button below the drop-down menu in the **Normalization** panel.

Alternative procedure:

1. Activate the reference dot definition mode by clicking the **Reference Dot Selection** tool on the **Normalization** panel.
2. Then select a spot in the **Images** window

To remove a previously assigned reference flag from a spot:

1. Click the spot(s) you want the reference flag to be removed.
2. Click the **Unref** button on the **Reference Definition** panel.

Alternative procedure:

1. Click the **Reference Dot Deselection** tool on the **Normalization** panel.
2. Then select the spot in the **Images** window you want the reference flag to be removed.

Reference spots can by marked by color or label. Just specify the marking by selecting one of the **Mark by** radio buttons.

Grid Reference Dots

Using the *Grid Reference Dots* method, each sub-grid is normalized individually.The reference value for a sub-grid results from averaging the integral or integral-bkg values of all reference spots in the sub-grid.

Data Range

Data Range represents an additional normalization method, where guide dots, empty dots and dots with low/high signal intensity can be excluded from referencing.

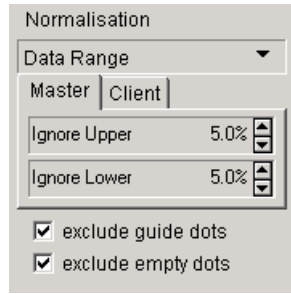


Figure 3-8 Data Range Normalization Method

Only the mid data ranges of the arrays are used for normalization purpose. These ranges are displayed in the **Quantil Plot** data view.

Data Range is a global normalization method, normalizing all spots using the same reference value.

Setting up and Reviewing Array Comparisons

In order to achieve meaningful comparison data, the settings for the background threshold (using the **Ratio Comparison** panel on the **Array Comparison** toolbox) and expression thresholds (data range spin boxes on the **Schematic Display** panel of the **Array Schematic** toolbox) have to be made properly.

Usually, you will review the statistics of the array comparison using the correlation plot displayed in the **Array Correlation** window.

In the correlation plot, the vertical and horizontal yellow lines indicate the background thresholds (vertical line: **Master Cut Off**, horizontal line: **Client Cut Off**), whereas the lines shown in non-yellow colors indicate the respective expression thresholds set by the data range spin boxes on the **Schematic Display** panel of the **Array Schematic** toolbox.

Two diagonal yellow-colored lines represent the **Max. Ratio** value (Max. Ratio and 1/Max. Ratio) selectable via the **Ratio Comparison** panel of the **Array Comparison** toolbox.

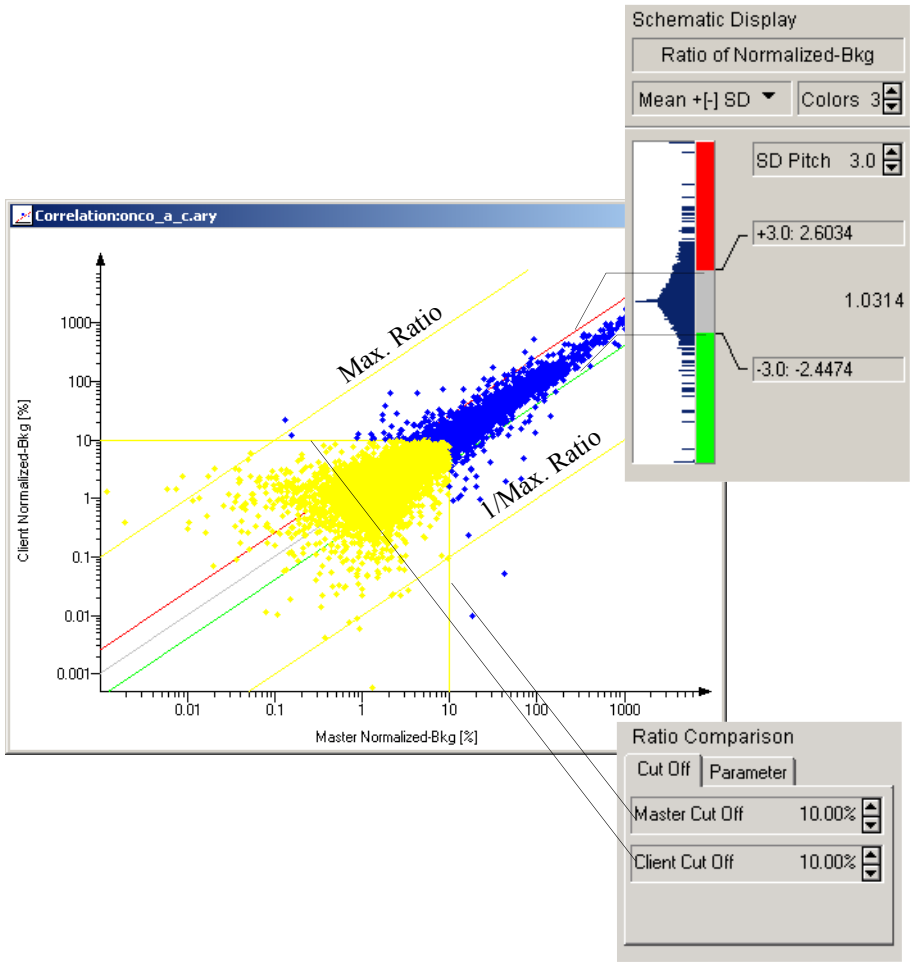


Figure 3-9 Correlation Plot Representation of Comparison Parameter

The background thresholds **Master Cut Off** and **Client Cut Off** divide the correlation plot into four parts:

1. Master value ≤ **Master Cut Off** *and* client value ≤ **Client Cut Off**: Both, master and client value are within the background range, i.e. their ratio cannot be determined. In this case, Array Compare sets the ratio value to **1** (expression value: **No**).
2. Master value ≤ **Master Cut Off** *and* client value > **Client Cut Off**: In this case, only the master value is within the background range, whereas the client value is above the background threshold. Their ratio cannot be determined, but since client's measurement value can be determined (in contrast to case 1 above), the ratio value is set to the predefined constant **Max. Ratio** (expression value: **On**).
3. Master value > **Master Cut Off** *and* client value ≤ **Client Cut Off**: This is the opposite case of 2., with the value of the master array above the background threshold and the client array value indeterminable.The ratio value is set to *1/Max. Ratio* (expression value: **Off**).
4. Master value > **Master Cut Off** *and* client value > **Client Cut Off**: Both values are above the background thresholds, so their ratio is determinable (= client value / master value). If ratio value > 1, the expression value is set to *ratio value*, and if ratio value < 1, the expression value is set to *-1/ratio value*.

Note – For values which are within the range of the expression thresholds (represented by the non-yellow colored lines, and determined by the data range spin box settings on the **Schematic Display** panel), the real ratio values are calculated in any case. This is due to the fact that small fluctuations in the measurement data causes large differences in interpretation, especially at the intersection point of the **Master Cut Off** and the **Client Cut Off**.
For values which are within the range of the **Max. Ratio** and *1/Max. Ratio* thresholds, the real ratio values are calculated and displayed in the **Ratio** column of the result table, but the **Expression** value is set to **On** or **Off**.

By default, the Threshold Cut Off value for both, master and client, is set in terms of multiplies of the Bkg deviation. This behavior can be change by selecting **explicit Threshold** from the **Parameter** tab of the **Ratio Comparison** panel and then changing the threshold values for the master and the client separately.

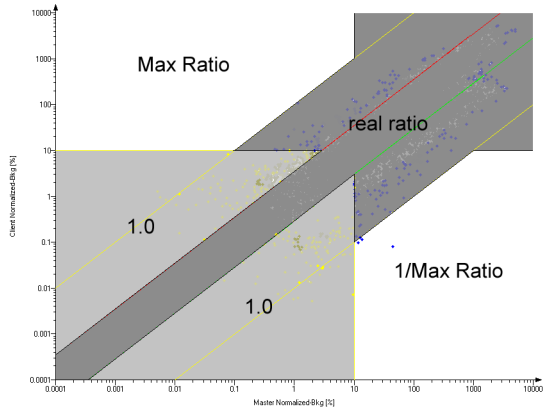


Figure 3-10 Correlation Plot Partitioned Into Different Ratio Calculation Areas

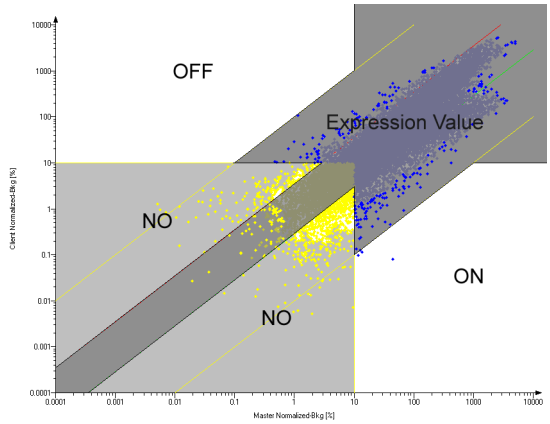


Figure 3-11 Correlation Plot Partitioned Into Different Expression Value Calculation Areas

Ratio Comparison – Example Procedure

1 Load master and client array as described in the “Loading Master and Client Arrays” section above.

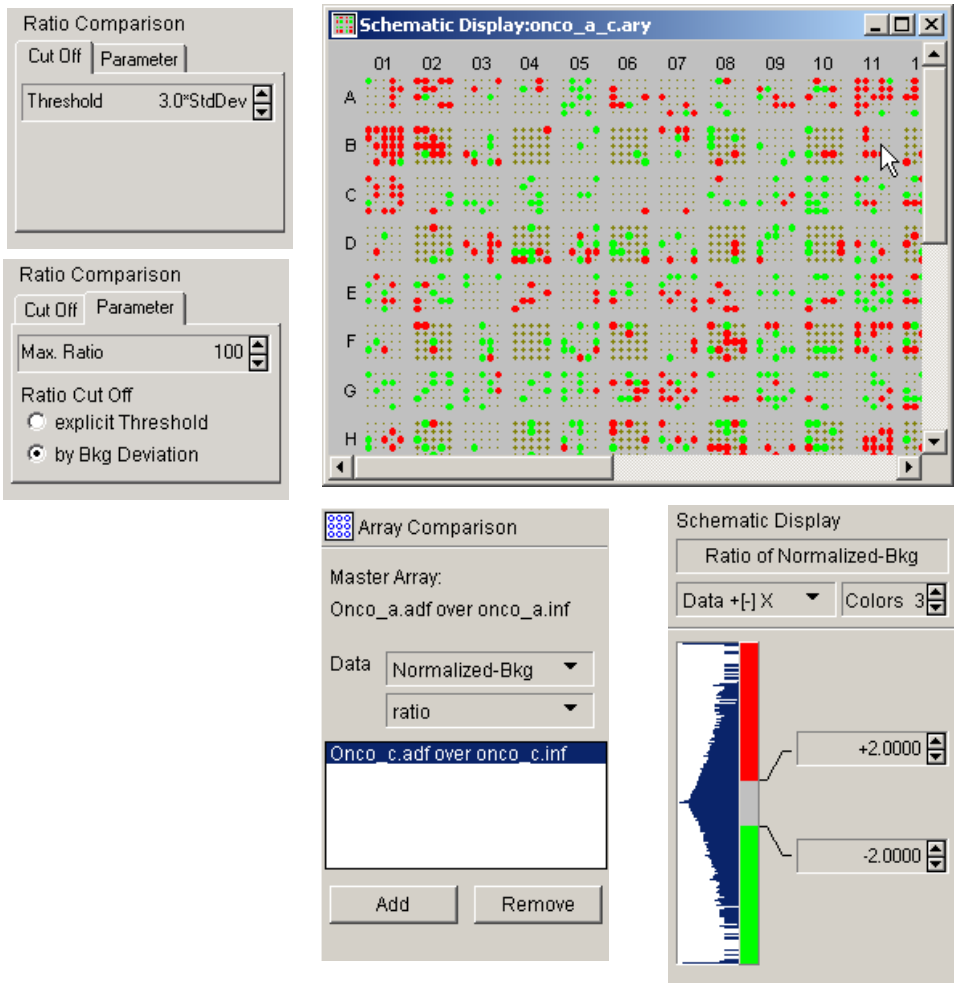


Figure 3-12 Schematic Display of Loaded Arrays With Toolbox Default Settings

Usually you do not have to change the default settings of the **Ratio Comparison** panel on the **Array Comparison** toolbox. They are suitable for most cases. But in some special cases, you might want to adjust the settings, following Step 2.

2 On the **Ratio Comparison** panel, select the **Parameter** tab, click the **explicit Threshold** radio button, and enter a value for **Max. Ratio**.

3 Select the **Cut Off** tab and set **Master Cut Off** and **Client Cut Off** to 5-10%.

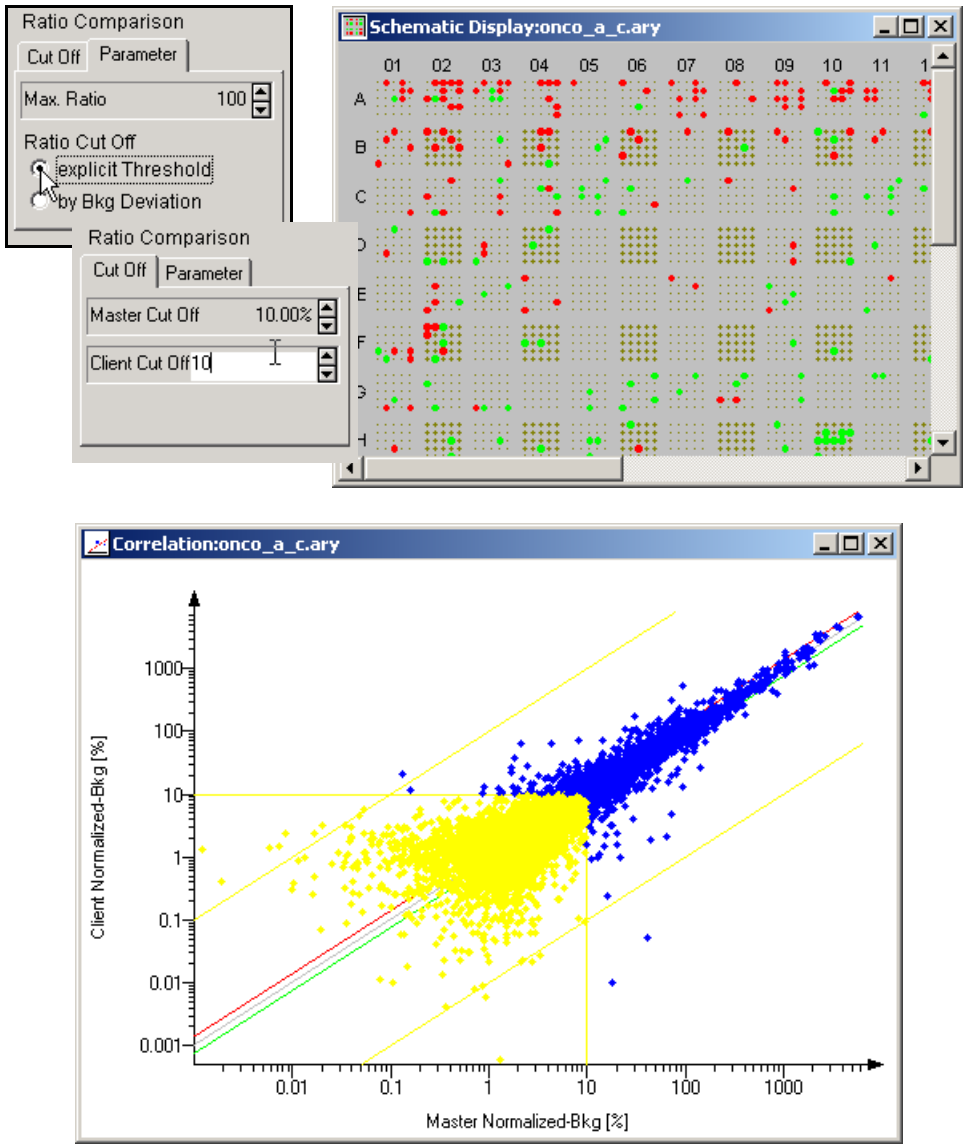


Figure 3-13 Schematic Display and Correlation Plot After Setting Background Threshold

Note that the number of colored dots in the **Schematic Display** window has considerably decreased.

4 On the **Array Schematic** toolbox, check the **Exclude from schematic** options for the dot types you want to exclude from the ratio comparison calculation and **Schematic Display** (as well as from the **Schematic Histogram** and the **Array Correlation** plot).

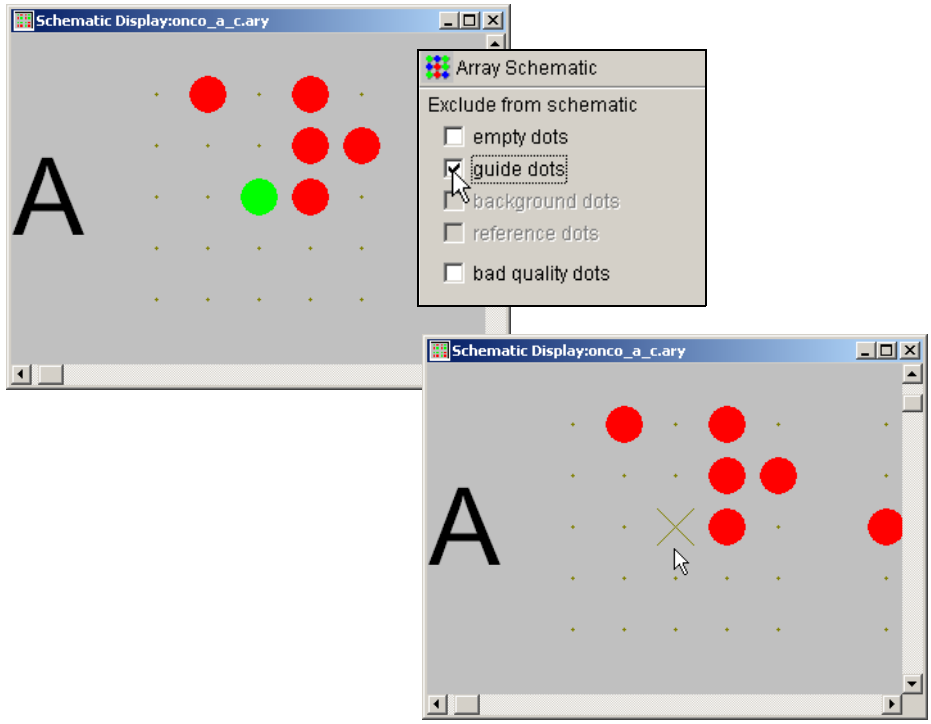


Figure 3-14 Excluding Dots

5 Increase the values for the expression thresholds via the data range spin boxes on the **Schematic Display** panel of the **Array Schematic** toolbox.

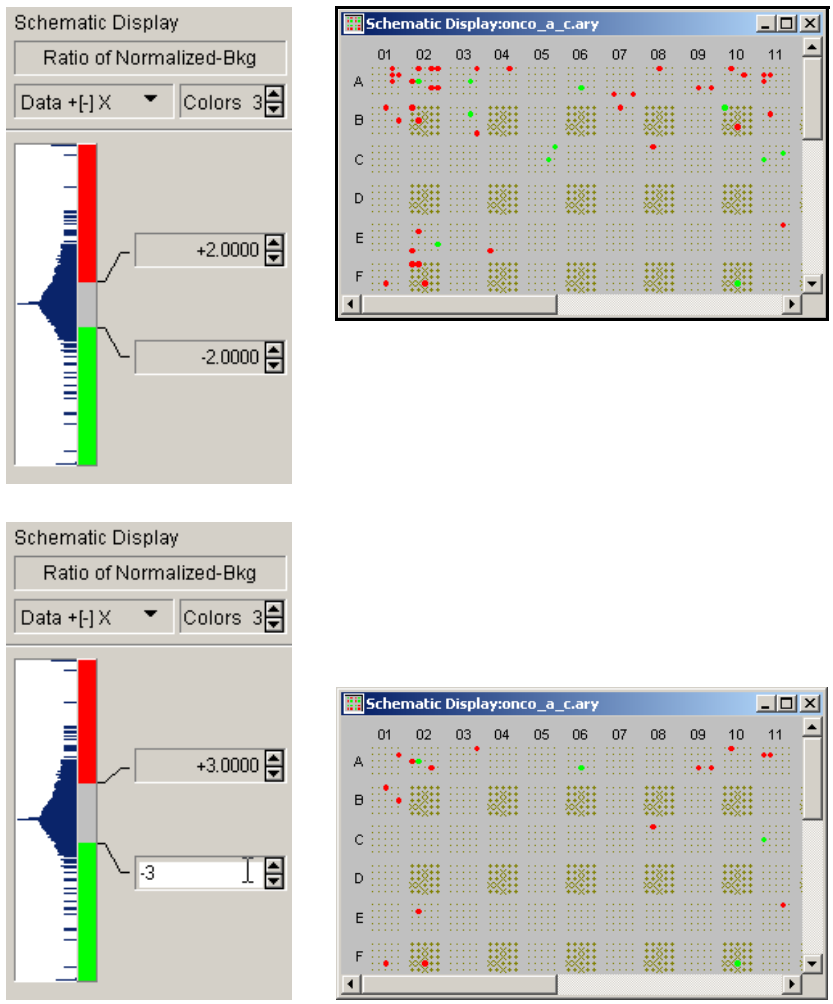


Figure 3-15 Schematic Display Before and After Increasing the Expression Threshold

6 Review the comparison representations and data utilizing the data window interlinkage feature.

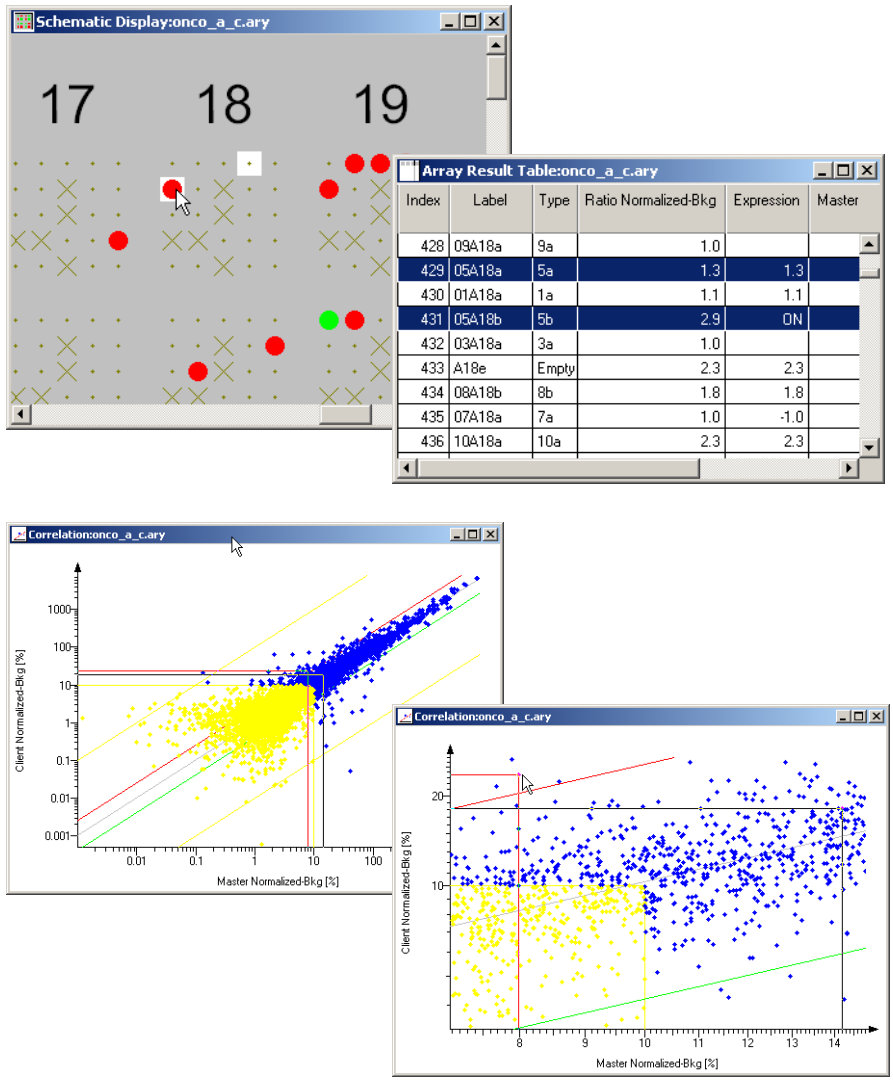

Schematic Display:onco_a_c.ary
17
18
19
Array Result Table:onco_a_c.ary
Index | Label | Type | Ratio Normalized-Bkg | Expression | Master
428 | 09A18a | 9a | 1.0 |
429 | 05A18a | 5a | 1.3 | 1.3
430 | 01A18a | 1a | 1.1 | 1.1
431 | 05A18b | 5b | 2.9 | ON
432 | 03A18a | 3a | 1.0 |
433 | A18e | Empty | 2.3 | 2.3
434 | 08A18b | 8b | 1.8 | 1.8
435 | 07A18a | 7a | 1.0 | -1.0
436 | 10A18a | 10a | 2.3 | 2.3
Correlation:onco_a_c.ary
Client Normalized-Bkg [%]
Master Normalized-Bkg [%]
Correlation:onco_a_c.ary
Client Normalized-Bkg [%]
Master Normalized-Bkg [%]

Figure 3-16 Reviewing Comparison Data and Representations

7 Finally, define two rules to include in the **Analysis Table** only those ratio values greater than or equal to 3 or smaller than or equal to –0.33 using the **Filter** panel of the **Array Analysis Table** toolbox.

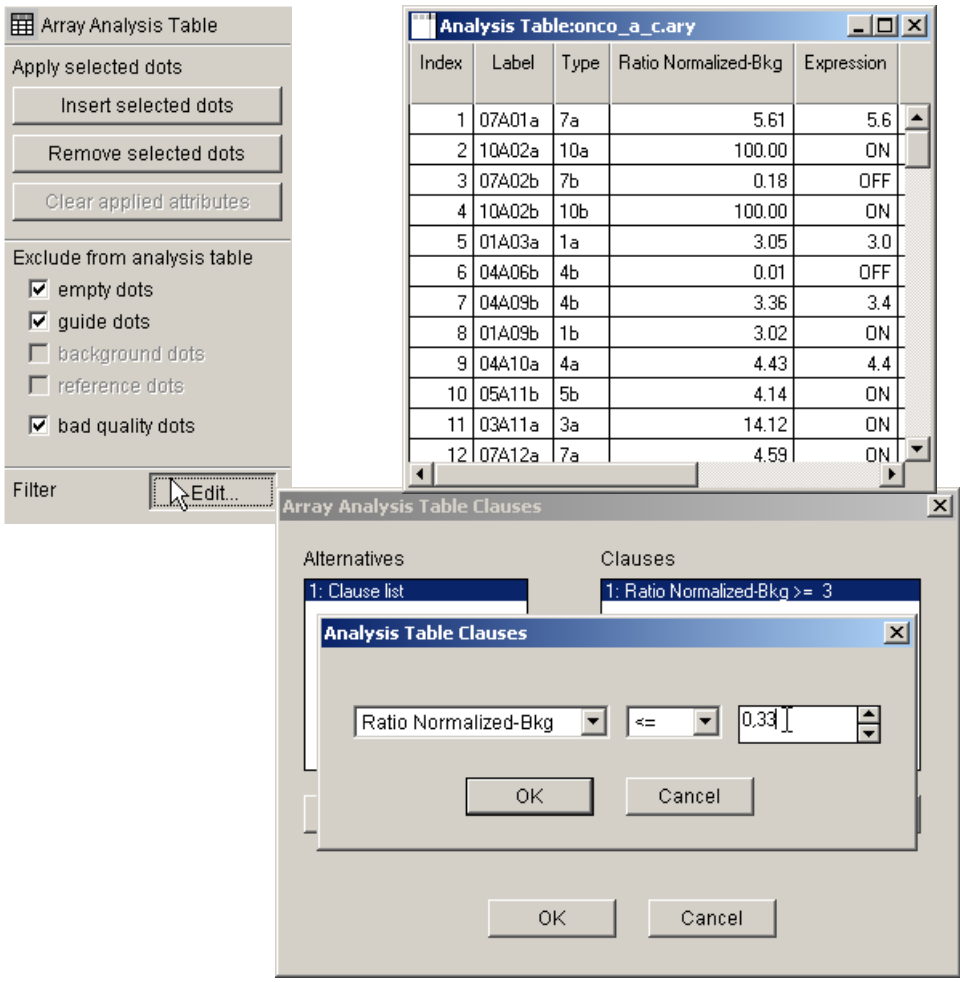


Figure 3-17 Defining Filter Rules

The resulting Array Analysis Table can be exported to a file for post-processing.

Appendix 4

Formal Description of Normalization

Let $Ref = (r_i | i = 1, \ldots, N\}$ denote the set of all reference clones within a filter. The normalization constant for a filter is determined by averaging the background corrected intensity values of the clones from these set.

$$C = \frac{1}{N}\sum_{i=1}^{N}(I(r_i) - B(r_i))$$

$I(r_i)$: Intensity value for clone r_i

$B(r_i)$: Background value for clone r_i

The normalized values for the clones are the relative signal values with respect to this constant.

$$N(clone) = \left(\frac{I(clone) - B(clone)}{C}\right)$$

These values are shown in the **Normalized-Bkg[%]** column of the Array Results Table in AIDA Array Metrix.

In AIDA Array Compare the comparison of two filters are now processed on the background corrected normalized values of the two filters, if you have selected the **Normalized-Bkg** values for comparison.

$$R(clone) = \frac{N2(clone)}{N1(clone)}$$

R(clone): Ratio value for a clone

N1(clone): Normalized background corrected value of clone in filter 1

N2(clone): Normalized background corrected value of clone in filter 2

Using the definition stated above, the formula can be rewritten by

$$R = \frac{N2}{N1} = \frac{\frac{I2 - B2}{C2}}{\frac{I1 - B1}{C1}} = \frac{I2 - B2}{I1 - B1} \cdot \frac{C1}{C2}$$

In Array Compare you can introduce expression values (2 and 0.5 for instance) for this ratio values to filter out and mark the up and down regulated genes.